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

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### 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 diver-

sity 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

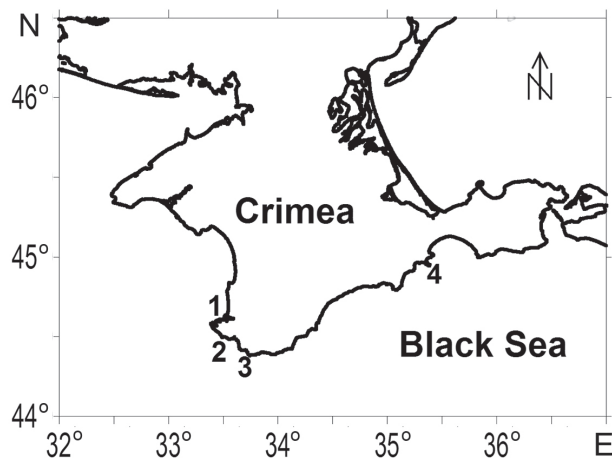
analysis of species richness between the previously studied coastal locations of South-western Crimea that suffer from various levels of anthropogenic pollution.

## 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 ( $S = 6.4 \text{ km}^2$ ) 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  $44^{\circ}59'09''$ , E  $35^{\circ}23'05''$ ) 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  $16 \text{ cm}^2$ . 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



**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.

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 ( $C_{\text{org}}$ ) 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).

Samples were initially treated in an ultrasonic bath for 20 min, then diatom valves were cleaned by HCl and  $\text{H}_2\text{SO}_4$  with addition of  $\text{K}_2\text{Cr}_2\text{O}_7$  (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 ( $S_{\text{exp}}$ ) 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   | Balakiava Bay        | Background level for coastal zone* | Background level for coastal zone** |
|---|---------------------|---------------------|--|----------------------|------------------------------------|-------------------------------------|
|   |                     |                     | Metals ( $\mu\text{g} \times \text{g}^{-1} \text{ dw}$ )           |                      |                                    |                                     |
| 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 ( $\text{ng} \times \text{g}^{-1} \text{ dw}$ ) |                      |                                    |                                     |
| 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, °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 <sub>2</sub> in near bottom layer, ml × l <sup>-1</sup> | 6,8                 | 7,4                 | 5,5 (4,8÷6,7)  | 6,5 (4,7÷8,2)        |                                    |                                     |
| C <sub>org</sub> , %                                      | 0,5                 | 0,5                 | 6,0 (2,6÷11,4)   | 2,4 (1,6÷3,0)        |                                    |                                     |
| PO <sub>4</sub> <sup>3-</sup>                             | 4,6***              | 2,2****             | 7,5 (2÷22)*****  | 8,1 (0÷92)*****      |                                    |                                     |
| NO <sub>3</sub> <sup>-</sup>                              | 7,2***              | 6,4****             | 27,6 (3÷86)*****   | 15,7 (0÷57)*****     |                                    |                                     |
| NO <sub>2</sub> <sup>-</sup>                              | 0,9***              | 0,7****             | 2,1 (0,4÷5,4)*****   | 1,3 (0÷6)*****       |                                    |                                     |
| NH <sub>4</sub> <sup>+</sup>                              | <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 intra-specific 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. *elipsoidea* 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 *Cocconeopsis* Witkowski, Lange-Bert & Metzeltin 2000 (Nevrova, 2015). Furthermore, the surveys also identified *Amicula speculum* (Witkowski) Witkowski (Fig. 4: 30), *Astartiella bahusienensis* (Grunow) Witkowski, Lange-Bertalot & Metzeltin (Fig. 2: 9; Fig. 4: 16), *Astartiella producta* Witkowski, Lange-Bertalot & Metzeltin (Fig. 4: 17), *Chamaepinnularia* sp. 1, *Cocconeopsis breviata* (Hust.) Witkowski, Lange-Bertalot & Metzeltin (Fig. 2: 3), and *Cocconeopsis 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.

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

*Continued*



Table 2 Continued

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

Continued

Table 2 Continued

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

Continued

Table 2 Continued

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

Continued



Table 2 Continued

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

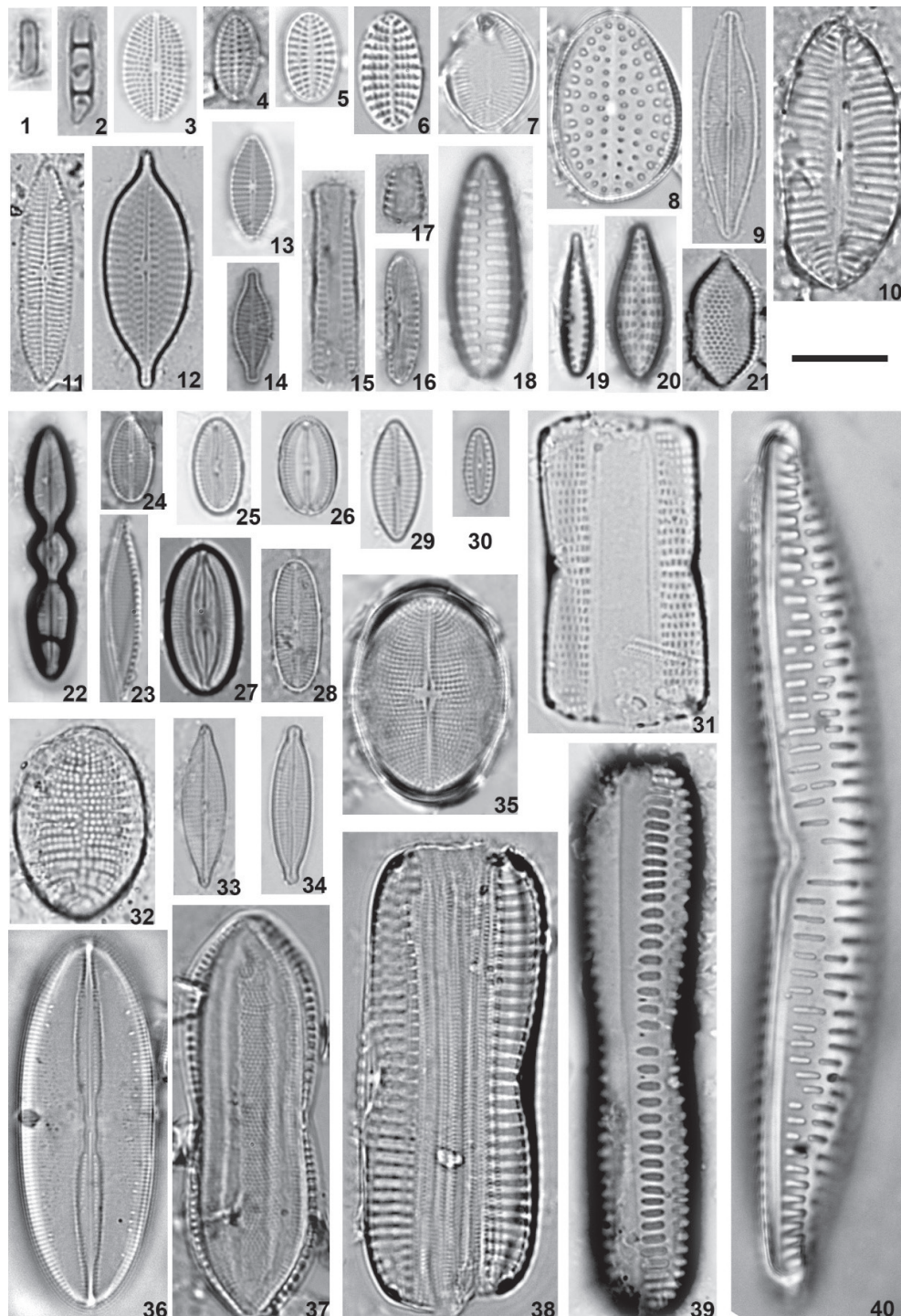
Classes Coscinodiscophyceae and Fragilariophyceae are represented slightly in the taxocene, at 7.2 and 6.9% of the total species number, respectively. From Coscinodiscophyceae, 5 orders, 9 families, 14 genera, and 22 species were registered, while 6 orders, 6 families, 13 genera, and 20 species were 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 Achnanthes (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*, *Fal-lacia*, 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 samples). 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 – *Cocconeopsis 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 amphiploides*; 26 – *Fallacia florinae*; 27 – *Fallacia oculiformis*; 28 – *Fallacia litoricola*; 29 – *Navicula alexandrae*; 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  $\mu$ m.

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

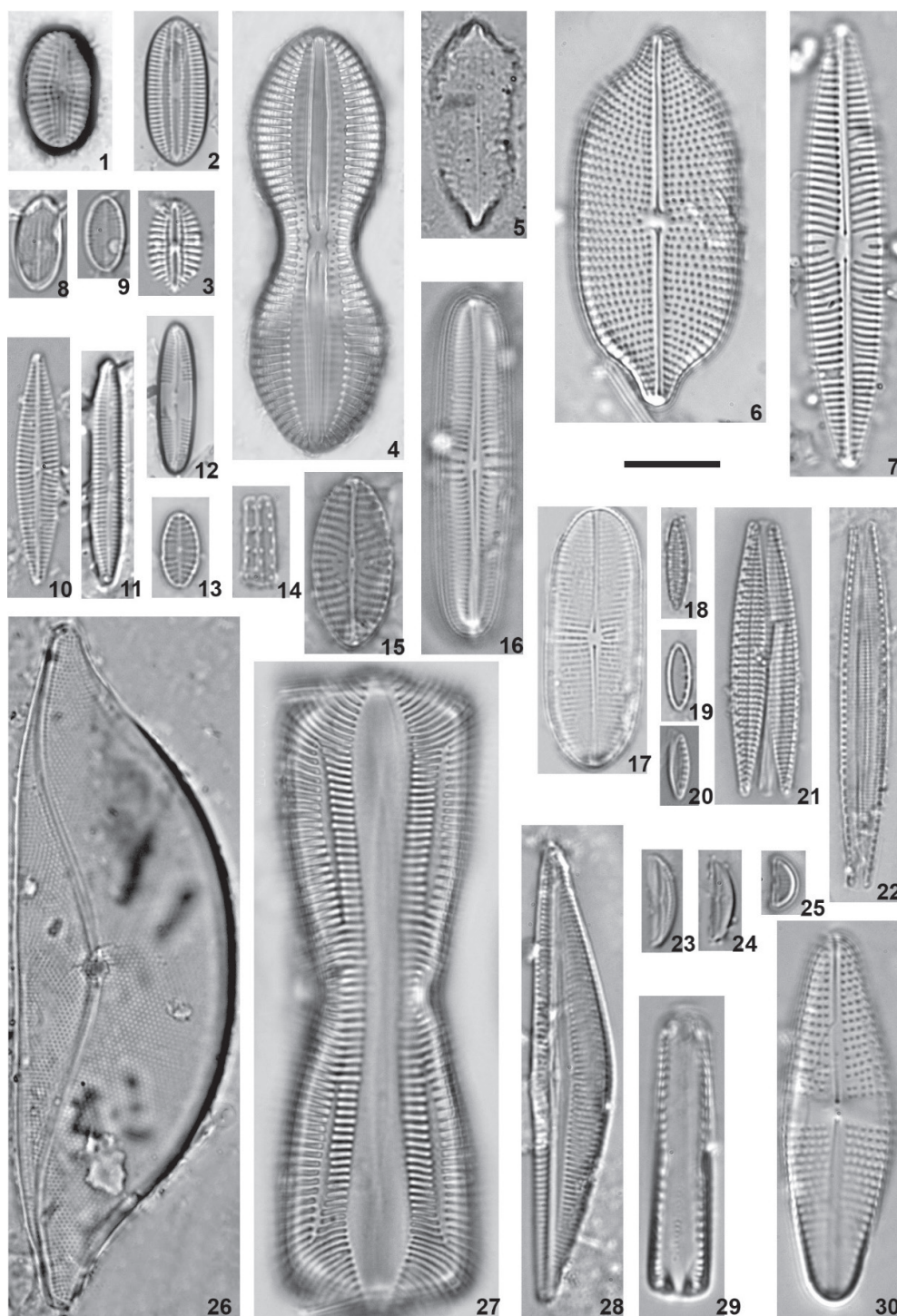
The high number of benthic diatom species registered in the DB may be due to this area not previously being subjected to anthropogenic disturbance. Levels of pollutants in this bay's soft bottom sediments were either close to the minimum analytical detection limits (for PAHs) or similar to average background 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 microhabitats 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 as 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 phytoplankton 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: *Achnanthes 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*, previ-

ously 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*, *Psammodyctyon 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 microhabitats 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          |





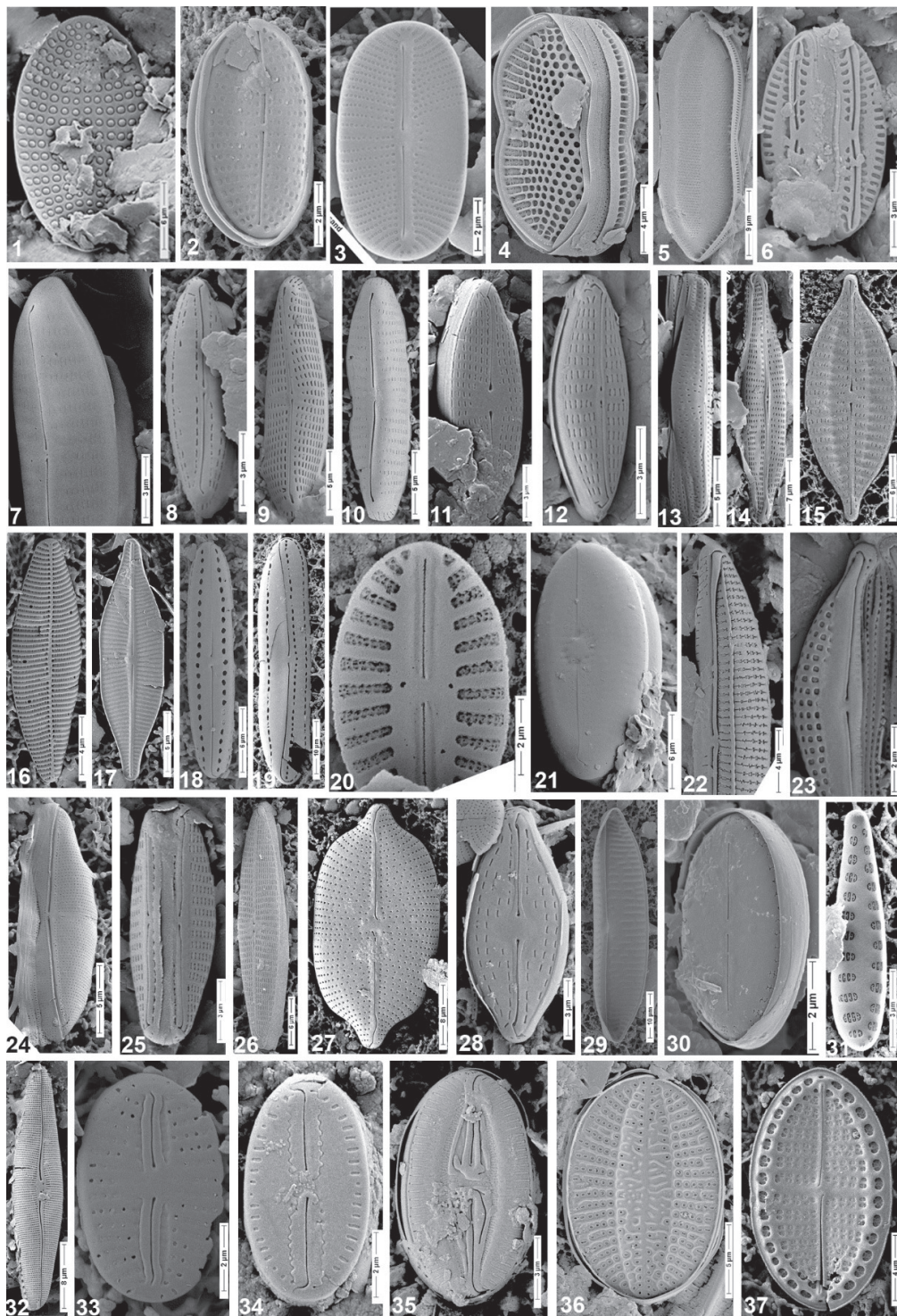
**Fig. 3:** New for the Black Sea diatom flora species found in Dvuyakornaya Bay (LM): 1 – *Diploneis* sp.1; 2 – *Diploneis parca*; 3 – *Diploneis* sp.2; 4 – *Diploneis stroemi*; 5 – *Navicula* cf. *opima*; 6 – *Navicula besarensis*; 7 – *Navicula erifuga*; 8 – *Cocconeis* sp.5W; 9 – *Cocconeis* sp.1; 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 liebethuthii*; 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  $\mu$ m.

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*





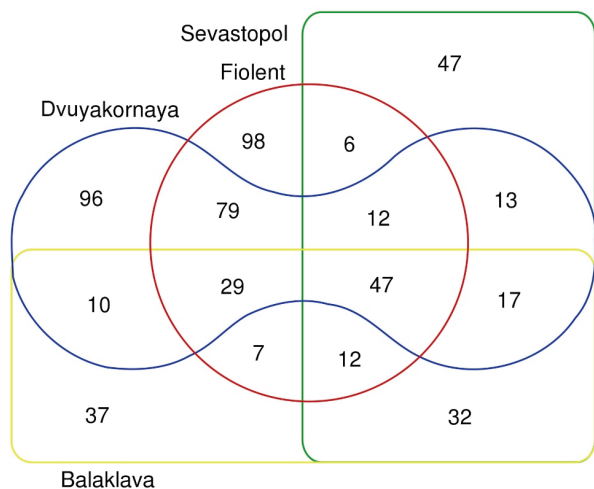
**Fig. 4:** New for the Black Sea diatom flora species found in Dvuyakornaya Bay (SEM): 1 – *Cocconeis guttata*; 2 – *Cocconeis diminuta*; 3 – *Cocconeopsis pullus*; 4 – *Psammodictyon panduriforme* var. *continua*; 5 – *Psammodictyon roridum*; 6 – *Amphora helenensis*; 7 – *Hippodonta* sp.3; 8 – *Hippodonta* 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.

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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).