Turkish Journal of Botany
http://journals.tubitak.gov.tr/botany/
Research Article
Turk J Bot
(2019) 43: 608-618
© TÜBİTAK
doi:10.3906/bot-1903-43

Assessment of benthic diatoms taxonomic diversity at coastal biotopes with different anthropogenic impact (Crimea, the Black Sea)

Elena L. NEVROVA*, **Alexei N. PETROV**
Benthos Ecology Department, Kovalevsky Institute of Marine Biological Research Russian Academy of Sciences, Moscow, Russian Federation

Received: 29.03.2019 • **Accepted/Published Online:** 07.06.2019 • **Final Version:** 06.09.2019

Abstract: An analysis of the taxonomic diversity of benthic diatoms was carried out for near-shore biotopes of Crimea with different content of trace metals and organic pollutants (PCBs, PAHs, DDTs). Taxonomic distinctness indices such as average taxonomic distinctness, AvTD (Δ^+) and variation in taxonomic distinctness, VarTD (Λ^+) were used for evaluation of the hierarchical structure of diatom taxocenes of two intact sites (Dvuyakornaya Bay and Cape Fiolent) and two heavily polluted water areas (Sevastopol Bay and Balaklava Bay). The highest similarity of species was revealed between healthy biotopes, in spite of their differences in hydrological and hydrochemical conditions. AvTD values for Dvuyakornaya Bay and Cape Fiolent ($\Delta^+ = 79.0$ and 76.7, respectively) were lower in comparison with expected average levels ($\Delta^+ = 82.2$) evaluated for the flora of benthic Bacillariophyta (1094 species) registered for the Northern part of the Black Sea (NPBS). As established, the taxonomic trees of the diatom taxocenes at pristine water areas are characterized by a moderate degree of vertical evenness and high variability of distances between neighboring clusters of species; they are mainly formed by polyspecific branches close on the genus level. The AvTD values for diatom taxocenes of the heavily polluted Sevastopol Bay ($\Delta^+ = 83.6$) and Balaklava Bay ($\Delta^+ = 84.1$) were significantly higher than the corresponding indices for undisturbed biotopes and exceeded the expected average level for the entire list of Bacillariophyta for NPBS. The upward trend of AvTD along the increasing technogenic impact can be caused by the disappearance of taxonomically close species with low tolerance from the same genus and prevalence of oligo- and monospecies branches in architectonics of the hierarchical tree. The present study shows that the application of TaxD indices can be considered an additional appropriate indicator tool for quantifying changes in the hierarchical structure of diatom taxocenes under persistent anthropogenic stress.

Key words: Benthic diatoms, Bacillariophyta, hierarchical structure, taxonomic indices, average taxonomic distinctness, variation in taxonomic distinctness, pollution, Crimea, Black Sea

1. Introduction

The key role of Bacillariophyta in the primary production and functioning of marine ecosystems necessitates the study of benthic diatoms both to preserve their biodiversity and to use them in assessing the state of marine environment (Van Dyke 2008; Blanco et al., 2012; Rimet and Bouchez, 2012; Borja et al., 2013; Winter et al., 2013; Stenger-Kovacs et al., 2014; Nevrova et al., 2015; Keck et al., 2016). Many coastal areas are subjected to increasing anthropogenic disturbance that causes significant changes in the species composition of benthic diatom taxocenes. High species richness and the complex taxonomic structure of diatom taxocenes in intact marine biotopes can change rapidly due to the fact that species that are sensitive to pollutants are eliminated and replaced by more tolerant ones.

The use of benthic diatom species was supposed to act as an early warning system in ecological monitoring. Due to being fast-reproducing organisms, most diatoms are widespread on different substrates and are closely associated with a certain microbiotope; they may promptly indicate adverse changes in main biotopic conditions determined by natural environmental processes and anthropogenic impact (Stenger-Kovacs et al., 2014). Therefore, comparative analysis of the structure of benthic diatom taxocenes in pristine coastal habitats and in anthropogenically perturbed biotopes is an important basis for identifying various aspects of the formation and sustainability of Bacillariophyta diversity under changing environmental conditions (Petrov et al., 2005; 2010; Facca and Sfriso, 2007; Heino et al., 2007; Petrov and Nevrova, 2007; Leira et al., 2009; Stenger-Kovacs et al., 2014; 2016; Nevrova, 2015). The importance of considering biodiversity indices at the interregional scale receives an increasing emphasis for planning the

* Correspondence: el_nevrova@mail.ru

conservation measures (Heino et al., 2007, Somerfield et al., 2009; Winter at al., 2013). Meanwhile, it should be noted that most marine ecological surveys of benthic diatoms focused on studying the seasonal dynamics of the leading species on hard substrates, and to a lesser extent on the study of soft bottom assemblages. The problems of diatom diversity assessment are very poorly investigated. Heterogeneous datasets, including historical data are often represented by a simple list of species without any values of biomass or number of individuals. In such cases, the use of traditional indices for measuring biodiversity (Shannon H, Margalef d, Pielou J, etc.), when both aspects of diversity are quantified according to the richness of species and evenness in the distribution of individuals between species, becomes impossible.

Most traditional biodiversity indices based on species richness can be strongly influenced by variability of environmental factors. Consequently, distinguishing the differences in the biotope caused by natural or anthropogenic changes can be recognized as one of the most difficult challenges for biodiversity monitoring (Leonard et al., 2006). Application of the above indices is also ineffective in cases when sampling efforts (number of samples) or habitat types differ significantly, as well as when comparing taxocenes with the same number of species and similar parameters of their quantitative development (Nevrova et al., 2015). Note that such taxocenes can include species that are phylogenetically close (belong to the same genus) or very distant (belong to different families, orders, and classes). In this regard, the taxonomic aspects of diversity can vary greatly even with the same species richness of the two compared taxocenes.

Application of hierarchical diversity measures recently developed by Warwick and Clarke in a form based on presence/absence data, i.e. average taxonomic distinctness (AvTD, Δ^+) and variation in taxonomic distinctness (VarTD, Λ^+), might represent a widely-applied tool for biodiversity study due to their particular properties (Warwick and Clarke, 1998, 2001). The AvTD index provides information on the relatedness of individuals on the taxonomic tree in a given community. The variance of AvTD is a measure that shows how a certain species is over- or underrepresented in a sample. The average taxonomic path length between two randomly chosen species (closely or distantly related phylogenetically) from the assemblage and value of its variance can correlate with anthropogenic impacts (Clarke and Warwick, 2001).

Taxonomic distinctness indices (TaxDI) are supposed to be more sensitive than quantitative diversity indices in discriminating among environmentally intact and perturbed biotopes since they incorporate not only quantitative data relating to species richness, but also particular information about taxonomic relatedness among species in comparing biotic assemblages (Somerfield et al., 1997; Warwick and Clarke, 1998; Arvanitidis et al., 2005; Munari et al., 2009). According to the calculation of TaxDI, the variability in biodiversity due to natural environmental factors generally falls within a predictable range based on the evaluation of expected TaxDI level corresponding to the regional species pool. Anthropogenic influences modify this pattern, such that diversity index can fall below the predicted range (Leonard et al., 2006).

A number of studies on different biotopes and taxonomic groups indicated that Δ^+ and Λ^+ can also alter in relation to gradients of natural influences (structure of substrates, hydrological intensity, nutrients level, etc.) that reduce the ability of the indices to distinguish anthropogenic perturbations from natural variability (Ellingsen et al., 2005; Mouillot et al., 2005; Bevilacqua et al., 2009; Vilmi et al, 2016). Such uncertain responses suggest the necessity of carrying out further study on the TaxD indices features in order to elucidate the anthropogenic effect on zoobenthic assemblages and diatom taxocenes. Regarding benthic diatoms, the application of TaxDI makes it possible to identify the differences in the taxonomic diversity related to changes in architectonics of hierarchical tree under different environmental conditions, even when analyzing historic datasets is represented in presence/absence format (as a list of species) (Clarke, Warwick, 2001; Ellingsen et al., 2005; Leonard et al., 2006; Price et al., 2006; Heino et al., 2007; Leira et al., 2009; Somerfield et al., 2009; Gottschalk and Kahlert, 2012; Rimet and Bouchez, 2012; Stenger-Kovacs et al., 2014; 2016; Nevrova et al., 2015).

Comparing the taxocene structure of diatoms (especially when considering the different hierarchical levels) can provide additional information concerning the influence of environmental fluctuations and anthropogenic impact on diatom diversity in different habitats. Note that until the last decade, the TaxDI index in marine diatom research, unlike other biota groups, was not investigated. Our works are the first attempts in marine diatomology to apply indicators of taxonomic distinctness to the quantitative analysis of hierarchical diversity of benthic diatoms dwelling in the coastal biotopes of the Black Sea. The study focused on comparing TaxDI values (as an indicator of the hierarchical structure of diatom taxocenes) for several Crimean coastal sites with different degrees of anthropogenic disturbance. Additionally, we analyzed the features of taxonomic trees architectonics driving the changes in the taxocene structure under comparison of intact vs. human-impacted habitats.

2. Materials and methods

2.1. Study areas

A comparative assessment of the benthic diatom hierarchical diversity using TaxDI indices was carried out in several nearshore areas of Crimea (the Black Sea) displaying different levels of anthropogenic disturbance. Among the studied locations were two pristine sites – Dvuyakornaya Bay and Cape Fiolent, and two heavily polluted water areas – Sevastopol Bay and Balaklava Bay (Figure 1, Table 1). Duplicate samples at every sampling point were taken by a diver from the upper (1–4 cm) layer of the soft bottom using a meiobenthic tube with a surface area of 16 cm^2.

Dvuyakornaya (means "two-anchored") Bay as an open type (S = 6.42 km^2) is surrounded from the northeast by Feodosia, and from the west by Cape Kiik-Atlama (see Figure 1). The steep coast consists of Jurassic conglomerates, clays, limestones and siderites (Guzhikov et al., 2012). Until the beginning of 2000s, the bay has been used as a military training area and was completely withdrawn from any industrial, municipal, and recreational exploitation. As a result, the natural conditions of the area were preserved. Currently, the shore is being developed with recreational facilities that may affect the ecological status of this water area and the state of benthic assemblages. The bottom in the bay consisted of silty-clay or sandy sediments covered with macrophyte fouling; at the depth greater than 10–12 m the silty substrate formed a continuous covering. A sampling survey was carried out in August 2008 at 11 points (N44°59′03″, E35°21′55″) within the depth range of 2–10 m.

The nearshore zone of Cape Fiolent extends about 10 km in length and is located in the Southwestern part of Sevastopol region (see Figure 1). The abrasion-bay coast, about 100 m high above the sea level, is composed of igneous (lava, tuff, keratophyre) and limestone rocks and is a remnant of an ancient volcanic eruption about 150 million years ago. Cape Fiolent is a natural example of modern evolution of ancient volcanic rocks in the marine zone; therefore, the landscape regional reserve "Cape Fiolent" was established here in 1996. The terrestrial part of the reserve area is 0.38 km^2, and the area of the adjacent waters of the Black Sea is 1.96 km^2. The seabed in the sampling area is formed from rocky outcrops with macrophyte fouling and patches covered with middle and coarse sand; bottom sediments deeper than 12 m consist of sand (Nevrova, 2016). Samples were taken in August 2009 (N44°30′53″, E33°30′28″) at 8 sampling points at the depth of 2–12 m.

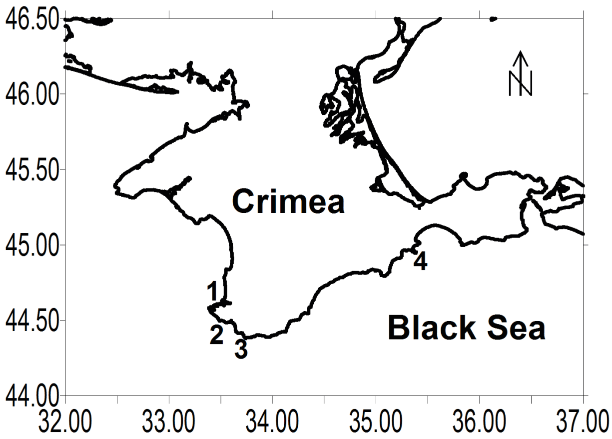

Figure 1. Scheme of sampling sites along the coast of Crimea (Black Sea). 1 – Sevastopol Bay, 2 – Cape Fiolent, 3 – Balaklava Bay, 4 – Dvuyakornaya Bay.

Balaklava Bay is a water area (S = 0.25 km^2) of intensive human disturbance with a permanent inflow of domestic and industrial wastewaters and fleet base activity products, which bring a wide range of pollutants into the environment (see Table 1). The accumulation of pollutants in bottom sediments of the bay have reached its highest intensity from the 1960s to the mid-1990s. The soft bottom in the sampling area consists entirely of silty sediments (Petrov et al., 2010; Nevrova, 2014). The sampling survey in Balaklava Bay was performed in September 2006 at 16 stations covering the entire bay water area (N44°29′44″, E33°35′39″) at the depth of 5–18 m.

Sevastopol Bay (S = 5.2 km^2) is also attributed to water areas of intensive human-driven usage, with a permanent flow of industrial and domestic wastewater (up to 15,000 $m^3 \times day^{-1}$), bringing many different pollutants into the bay environment (see Table 1). A hydrodynamic feature of this water area is the presence of an anticyclonic dynamic structure in the center of the bay, as well as the inflow of fresh water from the River Chernaya. Soft silty sand substrates evenly cover the entire bottom area of the main Sevastopol Bay (Petrov et al., 2005). Material was taken at 32 stations covering the entire bay at the depth from 4 to 17 m in July 2001 (N44°37′25″, E33°31′18″).

Note that in accordance with the result of a previous prognostic estimation (Petrov and Nevrova, 2013), nearly 80% of benthic diatoms species richness in the studied area can be revealed by analyzing the data from 6–7 stations. Thus, we can assert that in the studied sites of the Crimean coast, a quite thorough sampling survey of the taxonomic composition of Bacillariophyta assemblages was made.

2.2. Biological and statistical data analysis

Sampling surveys and treatment were performed at the Kovalevsky Institute of Marine Biological Research RAS, Sevastopol, Russia (IMBR). Sample processing for the cleaning of diatom valves followed the technique of cold burning in HCl and H_2SO_4 acids with the addition of $K_2Cr_2O_7$ (Nevrova et al., 2015). Taxonomic identification was carried out with a light microscope Nikon Eclipse E600 equipped with a PlanAPO 100× lens (Institute of Marine Sciences, Szczecin, Poland) and with scanning electron microscope Hitachi S-4500 (Goethe University, Frankfurt am Main, Germany).

Table 1. Mean concentration values of trace metals and 3 main classes of organic pollutants in soft-bottom sediments of Dvuyakornaya Bay, Cape Fiolent, Sevastopol Bay, and Balaklava Bay.

Metals, µg × g^{-1} dry weight									Organic pollutants, ng × g^{-1} dry weight			C_{org}, %
Cu (<20)*	Zn (<80)	Ni (<40)	Cr (<45)	Pb (<15)	Cd (<0.5)	Ag (<0.1)	Mn	Hg (<0.04)	PCBs (<50)	ChOPs (<1)	PAHs (<100)	
Dvuyakornaya Bay												
13.4 ± 0.2	98.4 ± 1.6	1.2 ± 0.1	2.5 ± 0.3	45.2 ± 1.7	0.03 ± 0.003	0.04 ± 0.01	638 ± 9	0.022 ± 0.001	31.2 ± 0.4	1.06 ± 0.12	<3	0.50 ± 0.06
Cape Fiolent												
22.8 ± 0.6	124.3 ± 1.4	5.5 ± 0.3	0.75 ± 0.05	21.8 ± 1.0	0.05 ± 0.003	0.02 ± 0.01	244 ± 4	0.021 ± 0.006	16.3 ± 0.4	1.10 ± 0.08	<3	0.50 ± 0.05
Sevastopol Bay												
97.9 ± 11.2	167.2 ± 15.8	34.0 ± 1.9	35.1 ± 2.8	96.1 ± 12.4	0.33 ± 0.07	0.30 ± 0.04	364 ± 40	1.118 ± 0.583	310.5 ± 48.2	15.93 ± 2.88	2713 ± 622	6.0 ± 0.4
Balaklava Bay												
161.4 ± 18.2	232.2 ± 32.8	30.8 ± 2.3	31.7 ± 3.8	338.7 ± 97.5	0.31 ± 0.04	0.34 ± 0.03	333 ± 22	0.811 ± 0.121	121.8 ± 27.1	18.80 ± 5.86	7054 ± 2017	3.1 ± 0.4

Notes: PCBs – polychlorinated biphenyls (sum of 4 congeners); ChOPs – chlorine-organic pesticides (sum of DDTs, DDD, and DDE); PAHs – polyaromatic hydrocarbons (sum of 16 isomers); C_{org} – total organic carbon. Measurements on study areas were kindly provided by colleagues from ICCWC NASU, Kiev.
* average background level of pollutants in surface sandy/muddy bottom sediments for the coastal zone of Crimea (in brackets) (Mitropolsky et al., 2006).
±: Standard error

Taxonomic identification was performed on the basis of related literature (Round et al., 1990; Witkowski et al., 2000, 2010, 2014; Levkov, 2009; Reid, 2012). Nomenclature of taxa was applied according to Fourtanier and Kociolek (Fourtanier and Kociolek, 1999, 2011). For classification of Bacillariophyta, the taxonomic system (Round et al., 1990) with additional information was used.

To provide a comparative analysis of structure and taxonomic diversity of benthic diatom taxocenes from investigated Crimean sites, a database of the Black Sea Bacillariophyta has been created using MS Office Access software. The database combines available publications as well as our own data; the scope includes Bulgarian, Romanian, Crimean, and Caucasian coasts, as well as the Northwestern shelf (Nevrova, 2015). The data from the Southern part of the Black Sea (coasts of Turkey) is still missing. The total list of species has been prepared according to the system outlined in Round et al. (1990) with the latest updates (Fourtanier and Kociolek, 1999, 2011; Levkov, 2009; Reid, 2012; Witkowski et al., 2000, 2010, 2014).

It should be noted that most of the data reported in the past century for the western part of the Black Sea contained only a list of species, without any microphotographs. Thereby there is a possibility that the same species in various publications of different authors have been indicated by different names and vice versa; several different species have been reported under a single name, taking into account the past broad concept in diatom taxonomy. However, we ought to use the historical results without an opportunity to verify the data on micrographs or slides, due to this we have no reason to distrust the published data of other diatomologists. Therefore, the data were updated and added into our database. As new works with micrographs of the Black Sea diatom species appear, our database will be updated and supplemented with new information.

The most comprehensive list of benthic Bacillariophyta of the northern part of the Black Sea without the coast of Turkey (NPBS), including 1094 species and intraspecific taxa (IST), was aggregated into 7 hierarchical levels (from IST to Division) (Nevrova, 2015). According to the taxonomic aggregation, the numerical values of Δ^+ and Λ^+ indices corresponding to the average expected level of the hierarchical structure of the NPBS diatom flora were calculated. A quantitative assessment of the hierarchical diversity of diatom taxocene in studied water areas was conducted using TaxD indices, where Δ^+ is the index of average taxonomic distinctness (AvTD) and Λ^+ is the index of variability (VarTD) (Warwick and Clarke, 1998, 2001). AvTD characterizes the vertical evenness of the taxonomic tree along ascending levels of hierarchy. VarTD reflects the horizontal asymmetry of the tree or the different representations of lower taxa in the higher taxa within individual ascending hierarchical branches (Warwick and Clarke, 1998, 2001; Nevrova, 2015; Nevrova et al., 2015). TaxDI calculations were carried out using the software package PRIMERv6 (Clarke and Gorley, 2006). In subsequent analysis, in order to visualize the regional differences in the taxonomic structure of the benthic diatom assemblages in relation to the average expected level for NPBS, the TaxDI values were superimposed on the plot, where the coordinate axes corresponded to AvTD and VarTD values. The values of Δ^+ and Λ^+ indices for the diatom taxocenes for each of the studied locations were defined. The points of Δ^+ and Λ^+ indices characterizing the hierarchical structure of the assemblage in sampling areas are located on the plot within the limits of bivariate ellipses, the center of which corresponds to the average expected value of TaxDI for the NPBS diatom flora. The boundaries of the ellipses correspond to the 95% probability contours of a "cloud" of the mean values of Δ^+ and Λ^+ calculated on the basis of 1000-fold random combinations for subsets of different numbers of species (50, 100, 300, etc.) withdrawn from a master list of Black Sea diatom flora. Previous studies showed that this algorithm allows the researcher to reliably assess the taxonomic diversity and to reveal features of the hierarchical structure of diatom taxocenes under different environmental disturbances, including technogenic pollution impact (Petrov and Nevrova, 2007; Nevrova, 2013, 2014, 2016; Nevrova et al., 2015).

2.3. Methods of chemical analyses

Chemical analysis of soft bottom samples was performed at the Institute of Colloid Chemistry and Water Chemistry, Kiev, Ukraine (ICCWC) following the techniques given in the literature (Burgess et al. 2009, 2011; Petrov et al. 2010). Analyses of organic and inorganic contaminants in sediments included measurements of 14 parameters: 9 metals (Ag, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Zn), total PCBs (4 congeners), total PAHs (as sum of 16 isomers), and pesticides (sum of DDT and metabolites). The content (%) of total organic carbon (TOC) and share of silt+clay fractions of the sediments were also measured. The sediment samples were prepared for analysis using ISO 11464:2006 standard methods. Samples were air dried, sieved, and homogenized. The content of metals (besides Hg) in sediments was determined by graphite (MDL 0.005–0.05 $\mu g \times kg^{-1}$ dry weight) and flame (MDL 2.0–15 $\mu g \times kg^{-1}$) atomic absorption spectrometry (AAS) following microwave digestion with a concentrated mixture of acids: HNO_3+HCl (3:1). Total Hg in sediments was determined by cold vapor AAS. Organic pollutants (PCBs and pesticides) in grounds were determined by GC/MS (MDL 1.0 $\mu g \times kg^{-1}$) using capillary column GC/ECD (MDL 0.05 $\mu g \times kg^{-1}$), following Soxhlet extraction with hexane/acetone (1:1) mixture. Total concentration of PCB homologues as the sum of tetra-, penta-, hexa-, and heptachlorobiphenyls was evaluated. Determination of PAHs was carried out by HPLC/UV (HP 1050/DAD, MDL 10–20 µg × kg^{-1}) in reversed-phase mode. Sediment grain size ratio of sandy, silty, and clay fractions (%) was measured by wet sieving and gravimetric sedimentation method.

3. Results

3.1. Pollutants in the bottom sediments of the studied areas

The content of the technogenic pollutants in soft bottom sediments of the Dvuyakornaya Bay and other coastal sites is reported in Table 1. As confirmed by multivariate statistical analysis before (Petrov et al. 2005; 2010), the combination of 10 key abiotic parameters, including 3 classes of organic pollutants (PCBs, PAHs, pesticides) and 7 metals (Cd, Cr, Cu, Hg, Ni, Pb, and Zn) attain the best match for the high similarities in the biotic and abiotic matrices, i.e. recognize a set of abiotic factors "best-explaining" the revealed differences in biotic parameters of the diatom taxocenes across the surveyed coastal area. Apparently, the combined effect of these environmental factors can lead to changes in species diversity features of the benthic diatom assemblages along with the extent of pollution in most of Black Sea coastal habitats.

Very low content of key trace metals and organic pollutants was registered in the bottom sediments of Dvuyakornaya Bay and near Cape Fiolent; the content hardly exceeded the average levels (numbers in brackets, see Table 1) of these pollutants in surface soft bottoms of environmentally healthy coastal areas of the Black Sea shelf (Mitropolsky et al., 2006; Burgess et al., 2009, 2011). The low level of pollution in sediments at Dvuyakornaya Bay and Cape Fiolent attributed these water areas to the category of conventionally pristine and characterized the structure of the diatom taxocenes in these biotopes as undisturbed. On the contrary, a considerable degree of pollution in Sevastopol Bay and Balaklava Bay (Burgess et al., 2009; Nevrova, 2014; Petrov et al., 2010) characterized these water areas as anthropogenically heavily disturbed (see Table 1). In comparison with pristine biotopes, the high level of accumulated pollutants (2–10-fold for most of the elements and up to 50 times higher for Hg and PAHs) can be influencing the structure of the benthic diatom assemblages and determines the changes in the TaxD indices values.

3.2. Floristic similarity analysis between the studied areas

Data on taxonomic representativeness of benthic Bacillariophyta in studied areas across 6 ascending taxonomic levels (from IST to order) is given in Table 2. In taxocenes of polluted biotopes, the species/order ratio was about 8/1 to 9/1, while for healthy sites this ratio was 15/1. Such differences indicate the predominance of oligospecies branches in hierarchical trees in the impacted bays, whereas in vertical taxonomic structure of taxocenes from pristine water areas, the polyspecific branches that are close on the genus hierarchical level prevail.

Results of comparative analysis of species composition (using the Bray–Curtis similarity coefficient) indicated that the highest similarity levels (57–58%) were observed between the taxocenes of Cape Fiolent and Dvuyakornaya Bay, and also between heavily polluted Sevastopol Bay and Balaklava Bay, despite the considerable spatial distance and differences in microenvironmental conditions in the compared areas. The smallest level of species similarity (32%–36%) was observed between the diatom assemblages of undisturbed and polluted biotopes (Table 3). Significant differences in diatom species composition may appear due to negative effects of pollutants, mainly upon low-tolerant forms.

3.3. Assessment of taxonomic diversity features of benthic diatom taxocenes

The taxonomic distinctness AvTD (Δ^+) and its variability VarTD (Λ^+) were calculated to compare the possible differences in hierarchical diversity features of taxocenes from the studied water areas (Figure 2). The values of the Δ^+ index for the taxocenes of Cape Fiolent ($\Delta^+ = 76.7$) and Dvuyakornaya Bay (79.0) were lower, while variability Λ^+ values were higher (360.4 and 345.9, respectively) than the expected TaxDI level ($\Delta^+ = 82.2$; $\Lambda^+ = 317.0$) calculated for NPBS Bacillariophyta flora (1094 species and IST). Lower values of AvTD indices compared with the expected level (center of the ellipses) indicated that taxocenes in pristine sites were formed mostly by taxonomically close species. On the contrary, the values of Δ^+ indices for taxocenes of polluted Sevastopol Bay (83.6) and Balaklava Bay (84.1) exceeded the expected value calculated for the master list. The Λ^+ index for Balaklava Bay (320.2) falls within expectation levels, whereas the Λ^+ value for Sevastopol Bay (268.6) was the lowest and went beyond the 95% probability contour of the expected average level (see Figure 2).

4. Discussion

Several studies discussed the effectiveness of both AvTD and VarTD indices in detecting variations in structure of taxocenes under different environmental perturbations, suggesting that these indices are quite well discriminate ecological alterations along the environmental gradients (Bevilacqua et al., 2009; Prato et al., 2009; Salas et al., 2006; Schratzberger et al., 2009; Stenger-Kovacs et al., 2014, 2016; Vilmi et al., 2016). It is known that compared to the average expected level of TaxDI, a lower degree of vertical hierarchical evenness of the taxonomic structure is typical for the communities exposed to prolonged adverse external influences, including technogenic pollution (Ellingsen et al., 2005; Heino et al., 2007; Gottschalk and

Table 2. Taxonomic representativeness of benthic Bacillariophyta in water areas of the investigated sites of the Crimean coast.

Class	Order	Family	Genus	Species	IST
Sevastopol Bay					
Coscinodiscophyceae	6	10	12	17	18
Fragilariophyceae	7	7	11	17	18
Bacillariophyceae	9	23	43	139	150
Total	22	40	66	173	186
Balaklava Bay					
Coscinodiscophyceae	5	9	13	25	26
Fragilariophyceae	8	8	12	20	20
Bacillariophyceae	8	21	37	138	145
Total	21	38	62	183	191
Cape Fiolent					
Coscinodiscophyceae	4	5	7	10	11
Fragilariophyceae	6	6	11	19	19
Bacillariophyceae	9	21	50	253	260
Total	19	32	68	281	290
Dvuyakornaya Bay					
Coscinodiscophyceae	5	9	14	22	22
Fragilariophyceae	6	6	13	20	21
Bacillariophyceae	9	22	51	257	261
Total	20	37	78	299	304

Table 3. Pairwise similarity (%) of benthic diatoms species composition in coastal biotopes 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	56.1	42.6

Kahlert, 2012; Petrov et al., 2010; Stenger-Kovacs et al., 2014). On the contrary, maximum values of the TaxD indices indicate a vertical evenness of the hierarchical structure, i.e. proportional representation of taxa at different hierarchical levels of the taxonomic tree (AvTD). High taxonomic variability (VarTD) is usually observed in environmentally undisturbed water areas (Keck et al., 2016; Leira et al., 2009; Nevrova et al., 2015; Rimet and Bouchez, 2012).

In our study we analysed several nearshore areas affected by different levels of anthropogenic disturbance that demonstrated pronounced differences in species composition. Low species similarity between pristine and impacted biotopes may indicate a strong influence of sediment pollution on taxocenes structure, especially upon forms with low tolerance disappearing in polluted biotopes (Sevastopol Bay and Balaklava Bay). Different levels of technogenic load in biotopes may modify the hierarchical patterns of benthic diatoms taxocenes from sandy/silty substrates. The results also confirmed the applicability of TaxDI evaluation approach to uncovering the alterations in hierarchical diversity of benthic Bacillariophyta related to different levels of pollutants on the bottom. The high species richness of benthic diatoms

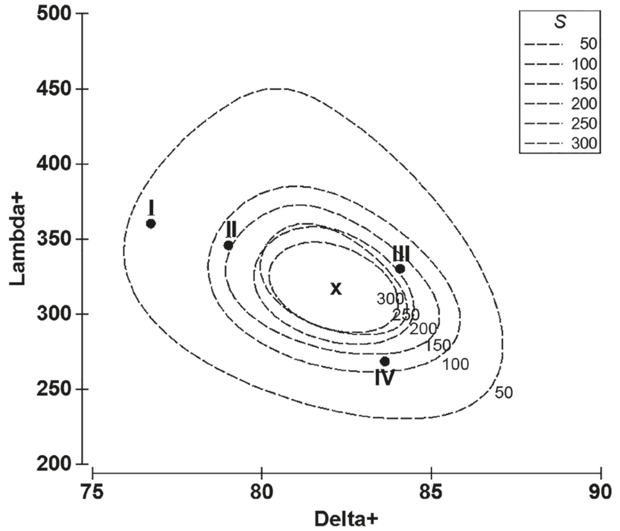

Figure 2. Points of taxonomic distinctness indices (paired values for Δ^+ and Λ^+) corresponding to four coastal sites of Crimea (I – Cape Fiolent, II – Dvuyakornaya Bay, III – Balaklava Bay, IV – Sevastopol Bay) superimposed on 95% probability bivariate ellipses calculated from 1000 independent simulations for several random subsets with different number of species (S = 50 … 300); × – center of TaxDI ellipses constructed from a master list of the NPBS benthic diatom flora (1094 species and IST).

revealed in Dvuyakornaya Bay (304 species and IST) and Cape Fiolent (290 species and IST) may have resulted from the fact that these biotopes are not subjected to any type of pollution (see Table 2).

Another possible reason influencing the formation of high species richness of Bacillariophyta in Dvuyakornaya Bay and near Cape Fiolent can be well-defined heterogeneity of microbiotopes (silty-clayed, rocky, or sandy substrates with mosaic patches of broken shells and macrophytes). A variety of bottom substrates and microniches might be favorable for the successful development of many diatom species, including ones highly sensitive to pollution as well as rare forms, alien species, and relicts of Ponto-Caspian flora.

The taxonomic tree of taxocenes from intact water areas is shaped by branches that include various numbers of species; such branches belong to different hierarchical levels (from species to order), but mainly form polyspecific taxonomic clusters closing up on the common genus level. The position of the point on the plot corresponding to diatom taxocene of Cape Fiolent is the most distant one from the center of the ellipse (see Figure 2). This fact suggests reduced vertical evenness and high variability of the taxocene structure, and indicates significant differences between the hierarchical structure of the taxocene at Cape Fiolent and the expected structure of the NPBS Bacillariophyta flora (Nevrova, 2016). The hierarchical structure of the taxocene in Dvuyakornaya Bay also displays a medium degree of vertical evenness and high variability compared with the expected level. However, according to other authors (Bevilacqua et al., 2011; Leonard et al., 2006; Prato et al., 2009), the most distant values of Δ^+ and Λ^+ indices that fall outside the average expectation levels can be observed in anthropogenically disturbed habitats, whereas in unpolluted biotopes under the influence of natural environmental factors only, values of TaxD indices fall within a 95% probability contour and are located close to the average expected level. The apparent differences in the taxonomic structure of taxocenes in studied areas may have been caused not only by the influence of external environmental factors, but also by internal aspects, such as divergent phylogenetic relationships of species. The absence of technogenic impact may lead to a relatively high appearance of taxonomically close species from the same genus. In such case the polyspecific branches begin to prevail in the hierarchical tree.

The diatom taxocene in the anthropogenically impacted Sevastopol Bay is characterized by relatively low species richness (186 species and IST). In the hierarchical structure dominated by oligospecies, branches that are close at the common genus level, along with the presence of a small number of mono-specific branches, are close on the family and order level. Such structural features, with reduced taxonomic variability between certain groups of branches, may reflect the changes in the diatom taxocene due to a considerable degree of pollution and low apparent substrate heterogeneity, where about 98% of the bay bottom area is covered with silty sediments (Nevrova, 2013). The responses of TaxD indices to technogenic disturbance imply that pollution could cause a distortion to the architectonics of taxocenes by reduction in the number of phylogenetically close species that are pooled within a high taxonomic rank (e.g., family or order) (Warwick and Clarke, 1998). It is noted that the extent of sensitivity or tolerance of diatom species to environmental stress can considerably vary even within the same genus. The structural features identifying the taxonomic level may also be connected with natural or anthropogenic perturbations in the biotope and influence taxonomic diversity metrics (Bevilacqua et al., 2011; Somerfield et al., 1997).

In heavily polluted Balaklava Bay, where bottom substrate is also homogeneous and represented by fine and silty sand, the structure of the diatom taxocene is expectedly similar to Sevastopol Bay. There is a reasonably low species richness (191 species and IST), a high ratio of mono- and oligospecies branches in the taxonomic tree, and in general, a moderate degree of vertical hierarchical evenness (Δ^+) (Nevrova, 2014; Petrov et al., 2010).

AvTD and VarTD are indices calculated based on presence/absence data; hence they could reduce the discriminating power when anthropogenic stress mainly affects relative densities of diatoms rather than the structure of taxocenes. A certain decrease in the sensitiveness of TaxD metrics also occurs when pollution has a pronounced effect on taxocene structure, but where a clear reduction in the number of species occurred in perturbed biotopes, it remained undetected when analyzing Δ^+ and Λ^+ indices (Bevilacqua et al., 2009; Leonard et al., 2006; Stenger-Kovacs et al., 2014, 2016). Thus, structural changes in taxocenes caused by anthropogenic disturbances (Sevastopol and Balaklava bays) could imply variations in taxonomic affinity among species that can be observed by architectonics of the taxonomic tree.

According to the results of hierarchical structure analysis of diatom taxocenes in the compared Black Sea coastal biotopes, it can be concluded that the formation of the taxonomic tree features depends on the altering of species richness due to emergence (or disappearance) of new branches that are close at different vertical levels of the taxonomic tree. Species that form new monospecies branches that are close at higher levels (family, order) appear (or are discovered by the researcher) in the taxocene structure less frequently than branches that are close at the genus level. The reverse process, i.e. the reduction of oligo- or polyspecific branches to monospecific ones leads to simplification of the hierarchical structure. It can also take place with a long term adverse impact on the taxocene (e.g., technogenic pollution) or due to insufficient knowledge on the biodiversity of the water area (Nevrova et al., 2015). The appearance (or disappearance) in the biotope of a sizeable number of new taxonomically close species (from the same genus) cause a much smaller influence on the features of the taxocene hierarchical tree and the TaxDI value than the input (or elimination) of even a few new species but with a distant phylogenetic relationship (Somerfield et al., 1997; Warwick and Clarke, 2001). Thus, the values of Δ^+ tend to decrease in relation to the average expected level when polyspecific closely related branches in the taxocene prevail.

5. Conclusion

Taxonomic distinctness indices provide more comprehensive insight into the architectonics of taxocenes, including in these metrics' elements related to the hierarchical relationships among species (or higher taxa) that are important for a deeper understanding of the concept of biological diversity. Our results revealed that the differences in the hierarchical tree of Black Sea benthic Bacillariophyta occurred under a different level of anthropogenic impact. The highest similarity of species composition structure of the hierarchical trees registered between taxocenes from highly polluted water areas (Sevastopol Bay vs. Balaklava Bay), as well as between conditionally pristine biotopes (Dvuyakornaya Bay vs. Cape Fiolent). Such affinity in hierarchical diversity features was found despite the geographical remoteness of these locations, differences in hydrological and hydrochemical conditions, and microlandscape heterogeneity of bottom substrates. The low similarity level of diatom species composition when comparing pristine and impacted biotopes may indicate a strong influence of sediment pollution on taxocenes structure, especially upon sensitive forms, usually disappearing in heavily polluted biotopes.

The values of AvTD for taxocenes from Dvuyakornaya Bay and Cape Fiolent were lower (79.0 and 76.7, respectively), and its variability (VarTD) was greater than the corresponding expected levels calculated for the NPBS Bacillariophyta flora. The taxonomic trees of diatoms are shaped by branches with a different number of species and are mainly formed by polyspecific taxonomic clusters close on the genus level.

The structure of diatom taxocenes in heavy polluted biotopes (Sevastopol Bay and Balaklava Bay) is characterized by higher values of the Δ^+ index (83.62 and 84.07, respectively) compared with the average expected level ($\Delta^+ = 82.2$). In the architecture of hierarchical trees, oligospecies branches dominate, along with the presence of a number of mono-specific arms that are close on higher hierarchical levels (family or order).

The AvTD and VarTD indices based on hierarchical taxocene structure of benthic diatoms might be recommended as an appropriate additional tool in the assessment of long term environmental disturbances in coastal marine habitats. Application of TaxD indices may be particularly suitable in cases when historical or modern datasets are represented in qualitative format only. However, the problem of effectiveness of taxonomic distinctness measures in disclosing the effects of technogenic impact upon biotic assemblages is still posing some questions regarding its application to various taxonomic groups of biota, including marine benthic diatoms. The solution to possible inconsistencies in discussed methodological issues requires further accumulation and improvement of knowledge on the structural responses of different microbenthos groups dwelling in various environmental conditions.

Acknowledgements

This study was funded by the Kovalevsky Institute of Marine Biological Research RAS state assignment project № 0828–2018–0002 (state registration № AAAA–A18–118020890074–2). This research was carried out in the Benthos Ecology Department of IMBR RAS. We express our appreciation to Dr. Sci. Prof. Andrzej Witkowski from the Institute of Marine Sciences, University of Szczecin, Poland and Dr. Sci. Prof. Horst Lange-Bertalot from Goethe University, Frankfurt am Main, Germany, for their help with taxonomical identification of diatoms. The authors express their appreciation to Dr. Sci. Anna Terletskaya and colleagues (Institute of Colloid Chemistry and Water Chemistry of NASU, Kiev) for the results of chemical analysis of sediment samples.

References

Arvanitidis C, Atzigeorgiu G, Koutsoubas D, Kevrekidis T, Dounas A et al. (2005). Estimating lagoonal biodiversity comparison of rapid assessment techniques. Helgoland Marine Research 59: 177-186.

Bevilacqua S, Fraschetti S, Musco L, Terlizzi A (2009). Taxonomic sufficiency in the detection of natural and human-induced changes in marine assemblages: a comparison of habitats and taxonomic groups. Marine Pollution Bulletin 58: 1850-1859.

Bevilacqua S, Fraschetti S, Musco L, Guarnieri G, Terlizzi A (2011). Low sensitiveness of taxonomic distinctness indices to human impacts: evidences across marine benthic organisms and habitat types. Ecological Indicators 11: 448-455.

Blanco S, Cejudo-Figueiras C, Tudesque L, Bécares E, Hoffmann L at al. (2012). Are diatom diversity indices reliable monitoring metrics? Hydrobiologia 695: 199-206.

Borja A, Elliott M, Andersen JH, Cardoso AC, Carstensen J et al. (2013). Good environmental status of marine ecosystems: what is it and how do we know when we have attained it? Marine Pollution Bulletin 76 (1): 16-27.

Burgess RM, Ho KT, Terletskaya AV, Milyukin MV, Demchenko VY 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 RM, Portis LM, Ho KT, Konovets IM, Petrov AN et al. (2011). Distribution, magnitude and characterization of the toxicity of Ukrainian estuarine sediments. Marine Pollution Bulletin 62 (11): 2442-2462.

Clarke KR, Warwick RM (2001). A further biodiversity index applicable to species list: variation in taxonomic distinctness. Marine Biology Progress Series 216: 265-278.

Clarke KR, Gorley RN (2006). PRIMER v6: user manual/tutorial, PRIMER-E, Plymouth.

Ellingsen KE, Clarke KR, Somerfield PJ (2005). Taxonomic distinctness as a measure of diversity applied over a large scale: the benthos of the Norwegian continental shelf. Journal of *Animal Ecology* 74: 1069-1079.

Facca C, Sfriso A (2007). Epipelic diatom spatial and temporal distribution and relationship with the main environmental parameters in coastal waters. Estuarine, Coast and Shelf Science 75: 35-49.

Fourtanier E, Kociolek JP (1999). Catalogue of diatom genera. Diatom Research 14 (1): 1-190.

Fourtanier E, Kociolek JP (2011). Catalogue of Diatom Names. California Academy of Sciences [online]. Fourtanier E, J.P. Kociolek JP, eds. http://researcharchive.calacademy.org/research/diatoms/names/index.asp [Accessed 19 September 2011]

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

Guzhikov AY, Bagaeva MI, Manikin AG (2012). New sedimentological, bio-, and magnetostratigraphic data on the Jurassic-Cretaceous boundary interval of Eastern Crimea (Feodosiya). Stratigraphy and Geological Correlation 20 (3): 261-294. (In Russian with an abstract in English).

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.

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

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

Leonard DRP, Clarke R, Somerfield PJ, Warwick RM (2006). The application of an indicator based on taxonomic distinctness for UK marine biodiversity assessments. Journal of Environmental Management 78: 52-62.

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

Mitropolsky OY, Nasedkin EI, Osokina NP (2006). Ecogeochemistry of the Black Sea. Kiev. (In Ukrainian with an abstract on English).

Mouillot D, Gaillard S, Aliaume C, Verlaque M, Belsher T et al. (2005). Ability of taxonomic diversity indices to discriminate coastal lagoon environments based on macrophyte communities. Ecological Indicators 5: 1-17.

Munari C, Warwick RM, Mistri M (2009). Monitoring with benthic fauna in Italian coastal lagoons: new tools for new prospects. Aquatic Conservation: Marine and Freshwater Ecosystems 19: 575-587.

Nevrova EL (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 with an abstract on English).

Nevrova EL (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 with an abstract on English). doi: 10.15407/alg24.01.047

Nevrova EL (2015). Donnyye diatomovyye vodorosli (Bacillariophyta) Chornogo morya: raznoobraziye i struktura taksotsenov razlichnykh biotopov: avtoref. dis. na soiskaniye uch. stepeni dokt. biol. nauk: spets. 03.02.01 – botanika, 03.02.10 – gidrobiologiya. Moskva. 2015. 46 s. (In Russian) [online]. Website: https://dlib.rsl.ru/01005555099 [Accessed 13 November 2014]

Nevrova EL (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 EL, Snigirova AA, Petrov AN, Kovaleva GV (2015). Guidelines for quality control of the Black Sea. Microphytobenthos. Simferopol: N. Orianda,. (In Russian with an abstract on English) doi: 10.21072/978-5-9907290-2-5

Petrov AN, Nevrova EL, Malakhova LV (2005). Multivariate analysis of benthic diatoms distribution across the multidimensional space of the environmental factors gradient in Sevastopol Bay (the Black Sea, Crimea). Marine Ecological Journal 3 (4): 65-77. (In Russian with an abstract on English).

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

Petrov AN, Nevrova EL, Terletskaya AV, Milyukin MV, 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.

Petrov AN, Nevrova EL (2013). Prognostic estimation of species richness of benthic Bacillariophyta. International Journal on Algae 15 (1): 5-25.

Prato S, Morgana JG, La Valle P, Finoia MG, Lattanzi L et al. (2009). Application of biotic and taxonomic distinctness indices in assessing the ecological quality status of two coastal lakes: Caprolace and Fogliano lakes (Central Italy). Ecological Indicators 9: 568-583.

Price ARG, Vincent LPA, Venkatachalam AJ, Bolton JJ, Basson PW (2006). Concordance between different measures of biodiversity in Indian Ocean macroalgae. Marine Ecology Progress Series 319: 85-91.

Reid G (2012). A revision of the family Pleurosigmataceae. Witkowski A (editor). Diatom Monographs. Ruggell, Liechtenstein: A.R.G. Gantner Verlag K.G. 14: 1-163.

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

Round FE, Crawford RM, Mann DG (1990). The diatoms. Biology and morphology of the genera. Cambridge, UK: Cambridge University Press.

Salas F, Patricio J, Marcos C, Pardal MA, Pérez-Ruzafa A et al. (2006). Are taxonomic distinctness measures compliant to other ecological indicators in assessing ecological status? Marine Pollution Bulletin 52: 817-829.

Schratzberger M, Lampadariou N, Somerfield PJ, Vandepitte L, Vanden Berghe E (2009). The impact of seabed disturbance on nematode communities: linking field and laboratory observations. Marine Biology 156: 709-724.

Somerfield PJ, Olsgard F, Carr MR (1997). A further examination of two new taxonomic distinctness measures. Marine Ecology Progress Series 154: 303-306.

Somerfield PJ, Arvanitidis C, Faulwetter S, Chatzigeorgiou G, Vasileiadou A et al. (2009). Assessing evidence for random assembly of marine benthic communities from regional species pools. Marine Ecology Progress Series 382: 279-286.

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 (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.

Van Dyke F (2008). Conservation Biology: Foundations, Concepts, Applications. 2nd ed. New York, NY, USA: Springer.

Vilmi A, Karjalainen SM, Kuoppala M, Tolonen KT, Heino J (2016). Taxonomic distinctness along nutrient gradients: more diverse, less diverse or not different from random? Ecological Indicators 61 (2): 1033-1041.

Warwick RM, Clarke KR (1998). Taxonomic distinctness and environmental assessment. Journal of Applied Ecology 35: 532-543.

Warwick RM, Clarke KR (2001). Practical measures of marine biodiversity based on relatedness of species. Oceanography and Marine Biology: an annual review 39: 207-231.

Winter M, Devictor V, Schweiger O (2013). Phylogenetic diversity and nature conservation: where are we? Trends in Ecology & Evolution 28: 199-204. doi: 10.1016/j.tree.2012.10.015

Witkowski A, Lange-Bertalot H, Metzelin D (2000). Diatom flora of marine coasts I. In: Lange-Bertalot H (editor). Iconographia diatomologica. Ruggell, Liechtenstein: A.R.G. Gantner Verlag K.G. 7: 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 JP (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. Beiheft 143: 469-484.