CONTRIBUTIONS TO THE SYSTEMATICS AND ECOLOGY OF THE BENTHIC DIATOMS OF THE ROMANIAN BLACK SEA LITTORAL

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The technique employed by the author in collecting and quantitatively processing the microphytobenthos from the mobile sediments is presented, and the list is given of the species new for the Romanian littoral benthos, some of which are at the same time new for the flora of Romania or of the Black Sea. The role of planktonic diatoms in the growth of the quantity of algae microflora on sediments is then analysed, as well as the relationship between the main ecological groups of the composition of microphytobenthos.

In the biological investigation of seas, the study of vegetable organisms, primary producers of organic substance, is of particular importance. In this sense, an altogether special attention is paid to phytoplankton, while investigations concerning the microphytobenthos are still sporadic and limited to small zones of the globe. The very investigation of the composition of benthal microflora is still insufficient, while as regards quantity, productivity, its significance in the trophic chain, extremely little is known about them. Nevertheless, the already existing data show clearly that in the infralittoral tier the microphytobenthos quantity [2], [5], [18], as well as its role in the production of organic substance [11], [12], and its participation in the trophic chain as direct food of many animals, among which fishes, too, [7], [9], [10], [13], [17], [19] are not in the least insignificant, but often greater than those of the phytoplankton. Even on sandy bottoms, which, due to the disturbance to which they are submitted by waves, might be considered as improper to the adaptation of diatoms, I found important amounts reaching up to 858 million cells/sq. m (August 1964, Mamaia, 12 m depth), figure which exceeds the most frequent density values of phytoplankton to 1 cu.m. water. Algae agglomerations covering boulders, known as foulings, contain downright impressive quantities, sometimes of several billion cells/sq. m [2], incomparable to those of phytoplankton, while amounts impossible to estimate, from the epibiosis of many macrophytic algae, in whose thickets many marine animals feed, easily convince us of the importance of this trophic link.

Our data prove that the investigation of neritic phytoplankton itself cannot be separated from that of the microphytobenthos. Over 35% of the species to be found in the composition of the phytoplankton of the shallow zone of the Romanian coast derive from the benthos [6].

It results clearly from the above that the significance of the microphytobenthos could not be overlooked. According to us, the relatively low level of knowledge, particularly as regards microflora from mobile bottoms — which occupy the widest surface of the infralittoral in all seas — is due to difficulties concerning processing technique, and even that of its collecting adequate to the quantitative study, in comparison to that of the phytoplankton.

In consideration of the practical necessity of studying this trophic link, we undertook — direct and guided by Prof. M. Băcescu, to whom we offer our warmest thanks —, the qualitative and quantitative investigation of microphytobenthos [1], [2], [5], parallelly with that of the phytoplankton of the zone between the coast and the 30 m isobath [1], [3], [4], [6]. We deem it necessary to make — prior to presenting new contributions to the knowledge of the composition and ecology of diatoms from the benthos —, some specifications concerning our working method.

In tackling this subject thorny problems were raised, some of which are ungraspable to begin with. The problem of collecting the material from sandy bottoms, which cover a surface of about 1000 sq. km of our littoral, proved necessary [1]. The difficulty of the problem consisted in the fact that sand does not adhere to the well tube, and the closing by valve is unpractical on hard bottoms causing their deterioration. In collaboration with a specialist, mechanical engineer M. Mercan, I conceived a heavy apparatus driven by an electrical winch, which collects by tippling sandy sediments in tubes each with a 1 sq. cm surface, inside which the natural stratification of the column is maintained (invention 48920, Bucharest, State Office for Inventions). Still by the same collaboration we conceived a well for quantitative collecting from silt bottoms. Until the manufacturing of apparata, samples were taken by direct immersion [2].

Materials were collected in each station from surfaces of 10 or 5 sq. cm, while the thickness of the sediment preserved — after previous observations concerning the depth in the sediment at which diatoms have a quantitative significance — is of 1 cm. Sediment fixing aboard was made with 4% formol.

For being investigated under the microscope, microflora has to be separated, as well as possible, from terigenous sediments. It is obvious that valid quantitative results can only be obtained if all, or at least the greatest number of cells to be found in sediments, are separated and picked out of the raw sample. If the separation of material from sandy sediments is simpler, and can be carried out by repeated decantation in water [2], this cannot be applied to silt bottoms, whose particles have densities close to those of diatoms (which, on an average, have the density of 2.1 [5]). For these sediments, as well as for those from fine sand, we used, almost without modifications, the method for fossil diatoms described by A. P.

Juze [14], which consists in principle in the separation of diatoms from sediments, by flotation in heavy fluids. The method requires many scrupulous manipulations, and much working time, particularly when series of a few samples are worked on; it yields, however, satisfactory results*).

For separation I used a solution of a mixture of cadmium iodide of a density of 2.3—2.45, through which, during centrifugation, sediments of greater density pass being deposited at the bottom of centrifuge phials, while the diatoms together with lighter parts of detritus and fine particles of silt, remain in suspension. The material extracted is then washed from the heavy solution by repeated decantations and centrifugations in distilled water, and afterwards again fixed in formol or ethylic alcohol. We mention that a centrifuge with large test tubes is absolutely necessary for introducing into them as much sediment and heavy liquid as possible. The volume of heavy liquid must be 3—5 times as great as that of sediment, in order that sand and silt particles be well dispersed in it, and carry with them, in their fall to the bottom of the phial, as little microflora as possible.

Processing under the microscope was made in conformity with the requirements of obtaining quantitative data for each species. Out of the volume of water with formol (as a rule of 10 cu. cm), in which the microflora from 10 or 5 sq. cm of substratum was concentrated, a fraction of 0.1 cu. cm was taken, from which live cells were counted by species, on the criterion of the presence of plasts, and the result of counting was referred to the surface of 1 sq. cm.

We deem necessary to point out the main difficulties encountered during the quantitative processing under the microscope:

Objectives with a magnifying capacity smaller than 40 ×, usually employed in counting by species in phytoplankton, are improper in the case of microphytobenthos, many of the small, hyaline diatoms are not merely undeterminable, but cannot even be observed. For counting by species, we therefore use objectives with a magnifying capacity of 40 × and 15 × oculars, which evidently extends the working time, the more so as in most of the stations, benthal microflora proves quantitatively rich and more diverse in species than the phytoplankton.

A great difficulty in counting by species occurs in connection with the fact that the only practical criterion of establishing whether the forms in the microscopic field were alive at the moment of fixation, is the presence of plasts, which, however, masks to a great extent the fine structure of siliceous valves. This demands that, in parallel, diatoms be examined without chromatophores, with a 100 × objective with immersion, on permanent preparations, in inclusion media, the measurement of frustules, and the counting of striae, spots, lineoles, being carried out, a. s. o. This part of the work is absolutely necessary, as determination indices, such as shape of cell or colony, number and aspects of chromatophores, utilizable in processing the phytoplankton, made up mainly of centrics, are absolutely improper for pennated diatoms, specific to benthos. The shape of many species, belonging to different genera, is the same, their great

*) We thank the staff of the Palinology sector of the Geology-Geography Institute of the Academy of the Socialist Republic of Romania, for having put at our disposal a working place and the necessary equipment in the period in which I started to work by this method.

majority not forming or not maintaining their colonies due to preparatory manipulations, while the aspect of chromatophores, often variable even in the same species, does not constitute a systematic criterion, in basic determinators being neither figured nor described. The number and disposition of fine elements of the valve structure is the main determination criterion, a large part of benthal forms not being easily related, at first sight, to one species or another, even if there is an experience in this sense.

Finally, another difficulty is due to the presence on preparation of parts of sediments and detritus which could not be removed by separation in heavy fluids and which cover parts of microflora.

Such difficulties which were, undoubtedly, encountered by other investigators interested in this problem, as well as the need of a systematic bibliography, as complete as possible, explain to the greatest extent the relatively backward condition we spoke about, as regards the international level of the knowledge in this domain.

We shall further present new data concerning the composition of the microphytobenthos. In an article published in 1964 [2] we gave the list of the benthal diatoms of the Romanian littoral, known to that date. It comprised 86 taxa, of which 6 determined merely as genus; the other 80 identified as species or variety, 5 (*Thalassiosira subsalina*, *Th. nordenskjoldii*, *Coscinodiscus perforatus*, *C. apiculatus*, *Thalassionema nitzschioides*) are typically planktonic. As forms with benthal life there remain 75 taxa. The list of systematic units newly found in the Romanian littoral benthos (table 1) comprises 122 taxa with benthal life and 9 taxa typically planktonic. The total number of taxa found in the Romanian littoral benthos, as it results from the summing up of the two lists, is of 211, of which 197 are diatoms with benthal life, and 14 planktonic forms sedimented on substratum.

Table 1

List of the species and varieties new to the Black Sea Romanian littoral benthos *)

a) Forms with benthal life:

	F	*Melosira distans* (Ehr.) Ktz.
	F	*Melosira granulata* (Ehr.) Ralfs
	M	*Melosira moniliformis* (O. Mull.) Ag. var. *subglobosa* Grun.
	M	*Melosira nummulodes* (Dillw.) Ag.
	M	*Podosira hormoides* (Mont.) Ktz.
x	M	*Podosira pellucida* Pr.-Lavr.
	M	*Hyalodiscus scoticus* (Ktz.) Grun.
	M	*Endyctia oceanica* Ehr.
x	M	*Actinocyclus ehrembergii* Ralfs var. *tenellus* (Bréb.) Hust.
x	M	*Biddulphia laevis* Ehr.
+	F	*Meridion circulare* Ag. var. *constrictum* (Ralfs) V.H.
	F	*Diatoma elongatum* (Lyngb.) Ag.

*) The present list completes the one published in 1964 (2), thus comprising only taxonomic units which were not mentioned then.

Signification of signs:

x = species new for Romanian flora; + species found for the first time in the Black Sea; M = marine and brackish water species; F = fresh water and fresh-brackish water species.

F *Diatoma vulgare* Bory var. *ehrenbergii* (Ktz.) Grun.
F *Diatoma vulgare* var. *linearis* Grun.
+ F *Diatoma vulgare* var. *producta* Grun.
F *Diatoma vulgare* fo. *subsalina* Pr.-Lavr.
x M *Dimerogramma minor* (Greg.) Ralfs
x F *Opephora marthii* Herib.
+ F *Fragilaria brevistriata* Grun.
F *Fragilaria capucina* Desm.
F *Fragilaria crotonensis* Kitt.
F *Fragilaria intermedia* Grun.
F *Fragilaria pinnata* Ehr.
F *Ceratoneis arcus* (Ehr.) Ktz.
M *Synedra gaillonii* (Bory.) Ehr.
F *Synerda ulna* (Nitzsch.) Ehr. var. *biceps* (Ktz.) Schönf.
M *Striatella interrupta* (Ehr.) Heib.
M *Striatella unipunctata* (Lyngb.) Ag.
M *Grammatophora oceanica* (Ehr.) Grun.
x M *Grammatophora serpentina* (Ralfs) Ehr.
x M *Licmophora abbreviata* Ag.
M *Licmophora ehrenbergii* (Ktz.) Grun.
x M *Licmophora paradoxa* (Lyngb.) Ag.
M *Cocconeis maxima* (Grun.) Per.
F *Cocconeis pediculus* Ehr.
F *Cocconeis placentula* (Ehr.) var. *euglipta* (Ehr.) Cl.
M *Cocconeis scutellum* Ehr. var. *parva* Grun.
F *Achnanthes lanceolata* (Bréb.) Grun.
+ F *Achnanthes lanceolata* var. *rostata* (Öestr.) Hust.
x M *Achnanthes manifera* Grun.
M *Rhoicosphaenia marina* (W. Sm.) M. Schmidt.
x M *Mastogloia binotata* (Grun.) Cl.
x M *Mastogloia paradoxa* Grun.
M *Diploneis bombus* Ehr.
x M *Diploneis chersonensis* (Grun.) Cl.
M *Diploneis littoralis* (Donk.) Cl.
x M *Diploneis notabilis* (Grev.) Cl.
x M *Diploneis notabilis* var. *tenera* Pr. Lavr.
x M *Diploneis ovalis* (Hilse) Cl. var. *oblogella* (Naeg.) Cl.
x F *Diploneis smithii* (Bréb.) Cl. fo. *rombica* Mer.
x M *Diploneis subadvena* Hust.
M *Amphipleura rutilans* (Trent.) Cl.
x M *Caloneis formosa* (Greg.) Cl.
x M *Caloneis liber* (W. Sm.)
M *Navicula abrupta* Donk.
x M *Navicula distans* (W. Sm.) Ralfs
x M *Navicula digitoradiata* (Greg.) Ralfs
x M *Navicula digitoradiata* var. *cyprinus* (Ehr.) W. Sm.
+ M *Navicula finmarchica* Cl. et Grun.
M *Navicula forcipata* Grev. var. *densestriata* A. S.
M *Navicula halophyla* (Grun.) Cl. var. *convergens* Pr.-Lavr.
M *Navicula hennedyi* W. Sm.

M *Navicula humerosa* Bréb.
\+ F *Navicula lanceolata* (A.G.) Ktz.
M *Navicula lyra* Ehr. var. *atlantica* A.S.
M *Navicula lyra* var. *ehrembergii* Cl.
M *Navicula lyra* var. *eliptica* A. S.
M *Navicula lyra* var. *intermedia* Perag.
x M *Navicula palpebralis* Bréb. var. *semiplena* Greg.
F *Navicula placentula* Ehr.
\+ F *Navicula placentula* fo. *rostrata* Mayer.
M *Navicula (Schizonema) ramosissima* Ag.
F *Navicula viridula* Ktz.
x M *Navicula directa* W. Sm.
x+ F *Pinularia major* (Ktz.) Cl. var. *paludosa* Meist.
M *Trachineis aspera* (Ehr.) Cl.
\+ F *Gyrosima acuminatus* (Ktz.) Rbh.
x M *Pleurosigma cuspidatum* Cl. var. *rostratum* Pr.-Lavr.
x M *Pleurosigma formosum* W. Sm.
M *Pleurosigma rigidum* W. Sm.
M *Tropidoneis lepidoptera* Cl.
M *Amphiprora alata* Ktz.
M *Amphora arcus* Greg.
M *Amphora granulata* Greg.
x M *Amphora granulata* var. *costata* Pr.-Lavr.
M *Amphora hyalina* Ktz.
x M *Amphora hyalina* var. *delicatula* Pr.-Lavr.
M *Amphora laevis* Greg.
x M *Amphora lineolata* Ehr.
x M *Amphora ostrearia* Bréb.
x M *Amphora proteoides* Hust. fo. *varians* Pr.-Lavr.
x M *Amphora proteus* Greg. fo. *ambigua* Pr.-Lavr.
x+ M *Amphora proteus* var. *laevistriata* A. Cl.
x M *Amphora proteus* var. *oculata* Per.
\+ F *Cymbella afinis* Ktz.
\+ F *Cymbella amphycephala* Naeg.
\+ F *Cymbella aspera* (Ehr.) Cl.
\+ F *Cymbella lanceolata* (Ehr.) V.H.
F *Gomphonema angustutum* (Ktz.) Rabh.
F *Gomophonema augur* Ehr.
F *Gomphonema olivaceum* (Lyngb.) Ktz.
\+ F *Gomphonema parvulum* Grun. var. *subellipticum* Cl.
F *Epithemia turgida* (Ehr.) Ktz.
\+ F *Epithemia turgida* var. *granulata* (Ehr.) Grun.
\+ F *Epithemia zebra* (Ehr.) Ktz. var. *porcelus* (Ktz.) Grun.
F *Rhopalodia gibba* (Ehr.) O. Mull.
M *Rhopalodia musculus* (Ktz.) O. Mull.
\+ F *Rhopalodia paralella* (Grun.) O. Mull.
x M *Bacillaria socialis* (Greg.) Grun. var. *baltica* Grun.
\+ F *Hantzschia amphyoxis* (Ehr.) Grun. fo. *capitata* O. Mull.
M *Nitzschia hybrida* Grun. fo. *hyalina* Pr.-Lavr.
M *Nitzschia lorenziana* Grun.

M *Nitzschia panduriformis* Greg.
M *Nitzschia panduriformis* var. *delicatula* Grun.
x M *Nitzschia punctata* (W. Sm.) Grun.
M *Nitzschia panduriformis* Greg. var. *minor* Grun.
x M *Nitzschia punctata* W. Sm. var. *elongata* Grun.
M *Nitzschia reversa* W. Sm.
M *Nitzschia tryblionella* Hantzsch. var. *debilis* (Arn.) Grun.
M *Surirella gemma* Ehr.
M *Surirella ovata* Ktz.
x M *Campylodiscus thurethii* Bréb. var. *lineolatus* Pr.-Lavr.

b) *Typical planktonic forms.*

F *Melosira italica* (Ehr.) Ktz.
M *Thalassiosira antiqua* (Grun.) Cl.
M *Thalassiosira excentrica* (Ehr.) Cl.
x M *Thalassiosira excentrica* var. *fasciculata* Hust.
M *Sceletonema costatum* Grun.
M *Thalassiosira parva* Pr.-Lavr.
M *Cyclotella caspia* Grun.
F *Cyclotella meneghiniana* Ktz.
F *Stephanodiscus astrea* (Ehr.) Grun.
— *Chaetoceros lorenzianus*, spores
— *Chaetoceros* spp., spores

Of the taxa of table 1, 41 (marked with an asterisk) are new for the flora of the Socialist Republic of Romania, too. At the same time, 19 taxa (indicated in the table with a +) are not mentioned in the Black Sea flora either, 17 being fresh water or fresh and brackish water kinds, thus possibly derived from the Danube or from littoral lakes. We therefore abstain from considering them as new for the sea flora, though — as we shall see further on — an important percentage of fresh and fresh-brackish water species are proper to the composition of the Romanian littoral microphytobenthos, as a consequence of the low salinity of the shallow waters, here. The two other species and varieties which, being marine, we consider new for the Black Sea benthal flora, are *Navicula finmarchica* and *Amphora proteus* var. *laevistriata*, forms known until now as Arctic [8], [15].

Of the 197 species and varieties of diatoms with benthal life, identified so far in the Romanian littoral benthos, only 15 (i.e. 7.6%) belong to the *Centricae* class. The other 172 species and varieties are of the *Pennatae* class, while of these the *Diraphineae* order represents alone 99 species, namely 50% of the total (table 2).

As regards the 14 species and varieties typically planktonic, 13 are related to the Centric ones (*Discinales* order) and one to the *Pennatae* (*Araphynales* order).

The presence on sediments of typically planktonic forms with unaltered chromatophores may be due to the following causes :

1. *The existence of a stage of rest in some neritic forms*, stages which permit them to survive on sediments in the unfavourable conditions of

their development in plankton. In algae cultures from sediments, the germination of several neritic planktonic species was obtained [16]. At this rest stage, species sporulate; in our materials we often found spores of the planktonic genus *Chaetoceros*. It is, however, possible that the rest stage may not necessarily imply the formation of spores, as some observations have already shown [16].

2. *The fall from the pelagial of planktonic microphytes*; this process occurs likewise in their normal vegetation period, but especially after death or at the end of the development period; when self-regulating functions of cells are either reduced or diminished. From our observations, the presence in appreciable amounts, on sediments, of planktonic species corresponds either to the flowering period, or to the immediately following stage, of the diminution of their amount in plankton.

3. *The transportation of planktonic microflora by vertical downward currents*. The intense hydrodynamism characterizing the Romanian littoral, favours by the mixture of waters on the vertical, the descent and sedimentation of microflora, just as it may also induce the reverse process of ascent into the pelagial of benthal microflora [6].

The fact that part of the planktonic cells may be dead, without having the entire possibility of recognizing them under the microscope, as the ceasing of vital functions does not bring about concomitantly the alteration of chromatophores [20], renders somewhat equivocal, the assessment of the precise proportion in which these species participate in the benthos. Nevertheless, the presence of some of them (*Thalassiosira parva*, *Th. excentrica* var. *fasciculata*, *Thalassionema nitzschioides*, *Cyclotella caspia*) with normal plasts in densities sometimes greater than many typically benthal forms (hundreds of cells to the sq. cm for each species), proves that their quantitative role in the benthos is not at all negligible. Thus, for instance, in February 1967, opposite Constantza, at the depth of 20 m, on a sandy bottom with fragments of shells, a total of 83 species was found, of which 74 (i.e. 86.75%) have benthal life, while 11 (i.e. 13.25%) are typically planktonic. Of the total of 7040 cells/sq. cm, the former amount to 5600 (i.e. 79.55%), and the latter to 1440 (i.e. 20.45%). In their turn, benthal forms participate, too, to the increase of the number of species and of the quantity of neritic phytoplankton, a fact presented at length in our works on phytoplankton [1], [3], [4], [6]. Thus, though well individualized, the two great cenoses are in a permanent interaction, some forms of one biotope passing into the other in considerable quantities.

In literature, besides planktonic and benthal species, a category of tichopelagic species is often delimited, whose life runs thoroughly normally, vegetating and being divided, both in the water mass and on its bottom. The typical planktonic forms indicated by us are not enclosed within this category. Of the marine and brackish water species, a large proportion of the *Discinales*, as well as certain pennated diatoms, such as *Amphiprora paludosa*, *Bacillaria paradoxa*, *Nitzschia closterium*, *N. tenuirostris*, a.o., are accepted as tichopelagic forms [20]. As regards the fresh water and fresh brackish water species found in our samples, most of them are considered as tichopelagic. We must observe, however, that particularly for the shallow zone, the acceptance of this category becomes somewhat artificial, as it is proved that besides the species included in it, many others indicated as proper only to one or another biotope, are to be found in both of them [1], [2], [3], [6], and as shown above, even in considerable densities.

The microphytobenthos may be considered as being almost exclusively constituted of diatoms. Only on boulders at small depths some representatives of the cyanophyceae (*Anabaena flosaque*, *Oscillatoria bonnemaisonii*, *Spirulina* sp.) and of the chlorophiceae (*Ankistrodesmus falkatus*, *Scenedesmus obliquus*, *Pediastrum boryanum*), fresh water and brackish water forms, are to be found. Likewise, besides marine planktonic diatoms, representatives of other groups of algae, frequent in the pelagial, such as *Exuviaella cordata*, *Prorocentrum micans*, *Glenodinium danicum*, *Gymnodinium* spp., *Ceratium tripos* — among perideneae —, *Coccolithus fragilis* — among coccolithophoridae —, *Dictyocha speculum*—among silicoflagellatae —, *Ebria tripartita* and *Hermesinum adriaticum* — among ebriaceae —, are to be found sporadically with the more or less normal plasts.

In conclusion we note the heterogeneous character of the composition by main ecological groups of the microflora of benthal diatoms (table 2), precisely as that of the planktonic one[6], character in which the mixture

Table 2

Composition by the systematic and ecological groups of the microflora of benthal diatoms

Order or suborder	Number of genera	Species and varieties		Ecological group: Marine and brackish water forms		Ecological group: Freshwater and fresh-brackish water forms	
		No.	%	No.	%	No.	%
O. Discinales	6	13	6.60	11	8.02	2	3.34
O. Biddulphiales	2	2	1.01	2	1.49	0	0.00
O. Araphinales	11	32	16.24	17	12.50	15	25.00
So. Monoraphineae	3	13	6.60	7	5.01	6	10.00
So. Diraphineae	14	99	50.26	72	52.55	27	45.00
O. Aulonaraphinales	7	38	19.29	28	20.43	10	16.66
Total	43	197	100.00	137	100.00	60	100.00

of waters of the zone close to the coast is reflected. Marine and brackish water species are represented by a percentage of 69.55%, while the fresh water and fresh-brackish water ones by 30.45%. The high proportion of fresh-water and fresh-brackish water species reflects, at the same time, the freshened brackish water regime of Romanian coast water (with salinities comprising between 8.61 and 18.67 g S‰. [21]) and the strong influence of the Danube in this portion of the sea.

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