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# GOLDEN ALGAE (CHRYSOPHYTA) BIODIVERSITY OF THE MOUNTAINOUS CRIMEA AND UKRAINIAN CARPATHIANS

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As a result of algological studies of different types of water bodies in the Crimean Mountains and the Ukrainian Carpathians, 144 Chrysophyta species were identified, represented by 154 taxa of intraspecies rank, including the nomenclature type of the species. For the first time, 82 (90) species are indicated for the golden algae flora of Ukraine, including three species described as new to science: Dinobryon emoriens, Kephyrion perforatum, Mallomonas montana. Ecological and geographical features, species composition, abundance and systematic structure of chrysophytes were established, as well as their uneven distribution in water bodies, which manifests itself in a decrease in species diversity in the following sequence: swamps  $\rightarrow$  lakes  $\rightarrow$  ponds  $\rightarrow$  rivers.

Key words: chrysophytes, biodiversity, algoflora, species composition, systematic structure, autecology.

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### БІОРІЗНОМАНІТНІСТЬ ЗОЛОТИСТИХ ВОДОРОСТЕЙ (*CHRYSOPHYTA*) ГІРСЬКОГО КРИМУ Й УКРАЇНСЬКИХ КАРПАТ

У результаті альгологічних досліджень різнотипних водойм Гірського Криму й Українських Карпат виявлено 144 види Chrysophyta, представлених 154 таксонами внутрішньовидової рангу разом із номенклатурним типом виду. Вперше для флори золотистих водоростей України наведено 82 (90) види, зокрема три види описано як нові для науки: Dinobryon emoriens, Kephyrion perforatum, Mallomonas montana. Визначено еколого-географічні особливості, видовий склад, чисельність і систематичну структуру хрізофітів, а також їх нерівномірний розподіл у водоймах, що проявляється зменшенням видового різноманіття в такій послідовності: болота — озера — ставки — річки.

Ключові слова: хрізофіти, біорізноманіття, альгофлора, видовий склад, систематична структура, аутекологія.

The choice of mountainous regions of Ukraine as regions for our research is explained by the almost complete lack of information about the chrysophytes of relevant physical and geographical regions. In addition, the conditions of aquatic ecosystems of mountain reservoirs coincide with the ecological peculiarity of golden algae – their cryophility, as well as their oligotrophy. The above stated the relevance of the work done.

The main purpose of the research was a critical study of the species composition and systematic structure of the golden algae of the Crimean Mountains and the Ukrainian Carpathians, establishing the features of their distribution in this area and in water bodies of various types. A detailed analysis of the results of the conducted studies made it possible to come to a number of important, with scientific novelty and practical value, conclusions.

Golden algae are a very polymorphous algae group that counts from 800 to more than 1000 species. As phototrophic organisms, they take part in the creation of the primary production of water bodies, serve as food for zooplankton and juvenile fish, thus entering the trophic chain. By dying off and settling on the bottom of the reservoir, chrysophytes algae take part in the formation of sapropel, which is widely used in the medicine and agronomy [1, 2, 10].

The main source of data on the species composition and distribution of golden algae in Ukraine remains the "Freshwater algae Key of the USSR" by A.M. Matvienko, which describes 526 species in 28 varieties and other forms. However, less than half of species listed in the determinant are found in the reservoirs of the vicinity of Kharkov (about 200), and the other half of the species (over 300) are submitted as potential for Ukraine.

The modern stage of golden algae research is associated with works devoted to the hydrobiological study of the lakes of Europe in general, and the flagellate algae of the mountain lakes of the Eastern Alps in particular, as well as to the chrysophytes of the lowland rivers [3, 7, 9]. An important source of modern information on classification, phylogenetics, taxonomy and the nomenclature of golden algae are a variety of databases that are posted on the Internet [8].

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**The purpose** of the study was a critical and systematic analysis of regional floras of Golden algae and determine their specificity in accordance with physical and geographical zoning, to find out the ecological and geographical features and the degree of occurrence of species of *Chrysophyta* in Ukraine.

**Materials and methods.** The material for this research was algological samples (784) collected by us during five expeditions and five excursions and also samples (650) collected by other collectors and stored in the algotheca of the Institute of Botany (National Academy of Science of Ukraine). In general, we studied the gold algae of 340 heterogeneous reservoirs. Samples were selected for ecological groups: neuston, plankton, periphyton and benthos.

Genus and species identification belonging to the majority of golden algae provides for the compulsory availability of living material for the treatment of which the optical microscopy methods of sealed preparations, hanging drops, floats and applications, sprouting cysts, and accumulation cultures were used [6]. The number of cells was determined using a Goryaev camera. Cumulative cultivation was carried out using the Michella nutrient medium for mobile and Richter medium for epiphytic forms. Making of preparations has several specific features. As a result of the analysis of literature data, as well as during own research, an original technique for preparing armored chrysomonades was developed for studying them with the help of a scanning electron microscope (JSM-35 manufactured by Jeol, Japan) [11].

To process the results of original studies, various parametric and nonparametric methods, including biometric analysis of species composition, floristic richness and taxonomic spectra, coefficients of frequency of occurrence, rank correlation, and floristic community are used. The data set was analyzed using the MS EXSEL (2016) Statistics & Math software package (versions 8 and 12; reliable interval 1-2%).

**Results of the study and their discussion.** For the first time a detailed study of the flora of golden algae of the Crimean Mountains (CM) and the Ukrainian Carpathians (UC). As a result of algological studies, 144 species were identified in different types of water in the study regions, which are represented by 154 taxa of intraspecies rank, which contain the nomenclatural type of the species (here and below the number of intraspecific taxa is given in parentheses). In particular, 82 (90) species were identified for the algal flora of Ukraine for the first time. Among them, 3 species are described as new to science: Dinobryon emoriens V. Nik., Kephyrion perforatum V. Nik. (fig. 1), Mallomonas montana V. Nik. Floristic wealth, systematic diversity and ecological features of the flora of golden algae in these territories are determined.



Fig. 1 Dinobrion emoriens V. Nik. species nova: a) a general view of the silica shell (SEM,  $3000^{x}$ ), b) the arrows indicate the micro-fibrils, which the silica shells are connected in a colony (SEM,  $4500^{x}$ ) & Kephyrion perforatum V. Nik. species nova: c) general view of the silica shell on the side (SEM,  $7200^{x}$ ), d) view of the shell to the top: perforations are visible (SEM,  $8600^{x}$ ).

The taxonomic spectrum of the flora of the golden algae of the MC is represented by one classis (Chrysophyceae), three ordo (Ochromonadales, Chromulinales, Stylococcales), 11 families, 28 genus and 76 (84) species. Becides, 81 taxa is indicated for the first time for this region, and 44 are new for Ukraine. The value of the randomness coefficient of the identified chrysophytes varieties ranged from 0.66 to 6.62. Its minimum values were noted for the majority of algae were found (60.6 %). During the analysis of the number of species that met "n" times (n=1, 2, 3), it was established that the number of species that met more than two times (n  $\in$  [3; 10]) was decreased (tab. 1).

Table 1

	number					
Classis	genus		species			
Ordo Familia	MC	UC	MC		UC	
	abs.	abs.	abs.	%	abs.	%
Chrysophyceae	28	27	84	100	116	98.3
Chromulinales	8	8	32	38.1	43	37.3
Chromulinaceae	2	1	13	15.5	7	6.1
Chrysococcaceae	2	3	12	14.3	25	21.3
Bicosoecaceae	1	1	1	1.2	2	1.7
Chrysamoebaceae	1	1	4	4.7	7	6.1
Kybotionaceae	-	1	-	-	1	0.9
Thallochrysidaceae	2	1	2	2.4	1	0.9
Ochromonadales	15	14	47	55.9	62	53.9
Ochromonadaceae	6	5	10	11.9	16	12.2
Dinobryonaceae	3	3	21	25.0	21	18.3
Synuraceae	4	4	12	14.3	25	21.7
Ruttneraceae	1	_	1	1.2	-	-
Phaeothamniaceae	1	2	3	3.6	2	1.7
Stylococcales	5	4	5	5.9	7	6.1
Stylococcaceae	5	4	5	5.9	7	6.1
Monosigales	-	1	-	-	1	0.9
Monosigaceae	-	-	-	-	1	0.9
Haptophyceae	-	2	-	-	2	1.7
Isochrysidales	-	2	-	-	2	1.7
Derepyxidaceae	-	2	-	_	2	1.7

Systematic structure and floristic richness of Golden algae of the Crimea Mountainous and the Ukrainian Carpathians

According to the number of identified species, the first were such genus as: Dinobryon, Chrysococcus, Mallomonas and Epipyxis. The main part of the generic spectrum is represented by 9 genus, which is 70 % of the total number of species registered for the flora of Chrysophyta MC and 32 % of genus. The main part of the spectrum of genus included five of the 11 identified ones: Dinobryon, Chromulina, Chrysococcus, Synura and Ocromonas, the total number of these species was 80 % from those which were found. At the same time, the overwhelming majority of species (94 %) belongs to two orders – Ochromonadales and Chromulinales.

As a result of analysis, the seasonal dynamics of species composition and population size, two groups were detected among the identified representatives of Chrysophyta. The first group, the most numerous, occurs in winter and spring. It included some species of genus Mallomonas, Chrysomoeba (Ch. radians), etc. The second group is occurred in the spring and autumn. It included all kinds of genus Chrysococcus and Thallochrysis (tab.2).

Depending on the typology, the distribution of golden algae along the CM's waters is uneven. The greatest number of species was found in the ponds and reservoirs (70). The second place in the species richness belongs to lakes (27). Then follow rivers and streams (15). An insignificant number of species are represented by chrysophytes springs (6), canals and ephemeral reservoirs (5 species each).

More than half of the detected golden algae in the CM water bodies (65.6 %) are represented by typical planktonic forms. The vast majority of them are active plankters with a monadic type of morphological structure of thaloma (representatives of the genus Mallomonas, Chromulina, Kephyrion, Pseudokephyrion, etc.). The other part leads an attached lifestyle (30 %). These include benthic (7 %) and periphytonic organisms (23 %).

Many of the species that were identified in the waters of the MC are organisms with pronounced cryophility (Chrysococcus diaphanus, Mallomonas elliptica, M. horrida, Kephyrion ovum, K. mastigophorum, etc.). The stenobionticity of chrysophytes is also confirmed by the acidophility of a large number of representatives of this group of algae. The part of the detected golden algae (20 %) in the MC

refers to acidophilic organisms. They were found at pH of water from 5.2 to 6.5 (Chrysamoeba mikrokonta, Ch. nobilis, Ochromonas fragilis, O. rholyphora, Lagynion notostomum, Heliochrysis eradians, etc.).

Table 2

in the waters of the Crimean stationary						
species	autumn	winter	spring			
Chromulina freiburgensis	$\leq 0.05$	-	0.25-0.65			
Ch. ovalis	-	0.25-0.65	0.25-0.65			
Chrysomoeba radians	-	0.25-0.65	0.65-1.20			
Chrysococcus diaphanus	0.05-0.25	-	0.05-0.25			
Ch. klebsianus	0.25-0.65	-	0.05-0.25			
Ch. rufescens	0.05-0.25	-	0.05-0.25			
Dinobryon cylindricum	-	0.05-0.25	0.25-0.65			
D. divergens	1.20-2.00	0.05-1.20	-			
D. sertularia	0.65-1.20	0.65-1.20	0.65-2.00			
D. sociale	-	-	0,65-2.00			
Epipyxi sutriculus	0.05-0.25	0.65-1.20	-			
Lagynion notostomum	-	0.05-1.20	-			
Mallomonas caudata	-	0.65-1.20	0.25-0.65			
M. fresenii	-	-	0.05-0.25			
M. gracillima	-	-	0,05-0.25			
M. parissae	-	-	0.25-0.65			
Microglena elliptica	-	0.65-1.20	0.05-0.25			
Ochromonas fragilis	_	0.25-1.20	0.05-0,25			
Thallochrysis pascheri	0.65-1.20	_	0.65-1.20			

#### Seasonal dynamics of species composition and abundance (10<sup>6</sup>cel./dm<sup>3</sup>) of chrysophytes that dominate in the waters of the Crimean stationary

In relation to the salinity of water, chrysophytes are stenohaline organisms like a majority of other algae. However, in the saltish Saki Lake (with a relative density of 1.030) was found a mesogalobic species of Wyssotzkia biciliata, and in one of the pons on the South Beach of Crimea (with a relative density of 1.010) was found the oligogalobic species of Chrysopyxis bipes.

In water reservoirs of the CM it was found 9 species of *Chrysophyta* from 32 species according to the list of organisms-indicators of saprobity that exists at the moment. Among them, one  $\chi$ -oligosaprobic species (Phaeodermatium rivulare), three oligosaprobic (Dinobryon sertularia, Epipyxis untriculus, Uroglena americana), two  $\beta$ -mesosaprobic (D. divergensi, Mallomonasac aroids) and three oligo- $\beta$ -mesosaprobic species (Chrysococcus rufescens, D. sociale var. stipitatum, Chrysosphaerella brevispina) were identified.

Analysis of values of the Pantle-Buck saprobity index showed that the water of the lake Laurel ("Cape Martyan Reserve") and several high-mountain lakes (the Crimean reserve-industrial economy) refers to the  $\chi$ -oligosaprobic zone. More than 20 reservoirs (springs, mountain watercourses and basins of the Nikitsky Botanical Garden – Crimean stationary) belong to the oligo- $\beta$ -mesosaprobic zones.

The taxonomic spectrum of the golden algae flora in the UC is represented by two classes (Chrysophyceae and Haptophyceae), five orders (Chromulinales, Ochromonadales, Stlococcales, Monosigales and Isochrysidales), 13 families, 29 genus and 108 (118) species (tab. 1). Among them, there are 108 species and intraspecies taxa described, included 45 new for algoflora of Ukraine for the first time in the region. And three species are described as new to science.

The value of the hit ratio of the identified species varied from 0.53 to 4.79. Its minimum values are noted for 55.9 % of the algae identified. A significant number of species that were found only once, may indicate a high level of specificity of the species composition of the golden algae of the UC.

The first places in the number of registered species took place the genus Mallomonas, Dinobryon, Chrysococcus, Kephyrion and Ochromonas. The main part of the generic spectrum is represented by 9 genus, constituting 76.5 % of the total number of species for the algoflora of Chrysophyta of the UC and 31 % of genus. The most complete were the genus Dinobryon, Chrysococcus, Chrysamoeba and Syncrypta. The head of the families spectrum included 6 detected families: Synuraceae, Chrysococcaceae, Dinobryonaceae, Ochromonadaceae, Chromulinaceae and Chrysamoebaceae. The total number of species families in the head part of the spectrum was 89,6 % of those that were found in the Ukrainian Carpathians. Moreover, the overwhelming majority of species (94 %) belong to two orders – Chromulinales and Ochromonadales.

The distribution of species by types of water bodies is uneven. The most number of species was found in bogs (66) and lakes (55). The third place in the species richness is occupied by ponds and reservoirs (52). Then follow rivers and streams (25), as well as ephemeral water bodies (18). An insignificant number of species (2) are represented by chrysophytes springs. The greater part (73.8 %) of the golden algae found in the reservoirs of the UC is represented by typical planktonic forms. Over 20 % of registered species have an attached lifestyle. These include 18 % epiphytic and slightly less benthic forms.

As mentioned earlier, many representatives of Chrysophyta are stenobiontes in relation to some abiotic factors. For example, a significant number of species (more than 25 %) are detected at temperature ranges of 3.4–10.0°C. Among them are explicit cryophilic species: Chrysococcus cordiformis, Kephyrion cupuliforme, K. densata, K. ovum, Pseodokephyrion poculum, Stephanoporos scherffelli, and most species of the Mallomonas genus.

About 60 % of chrysophytes that were found in the UC are recorded in their habitats with acidity of water, which varies between 5.3 and 6.5 (mainly in the bogs). The latter include characteristic acidophilic species: Ochromonas vagans, Synura echinulata, S. petersenii, S. sphagnicola, Kybotion eremita, Chrysamoe bamicrophaea, Ch. pyrenoidifera, some representatives of the genus Mallomonas, etc.

In relation to the salinity of water, the vast majority of chrysophytes algae that were identified in water reservoirs of UC are stenohaline species. However, there are data on the apparent halophobic nature of the species. In the various reservoirs of the UC, mainly in the trout ponds with a low level of total mineralization, four halophobic species have been found (Mallomonas coronifera, Chrysococcus rufescens, Ch. biporus, Ch. triporus). Moreover, 7 species are registered for this region, which are indifferent to this factor.

In the UC were identified 19 species of golden algae from 32 species of golden algae according to the list of organisms-indicators of saprobity. Among them, there are 8 oligosaprobic species (Chromulina rosanofii, Kephyrion moniliferum, Mallomonas coronifera, etc.), 5  $\beta$ -oligosaprobic (Pseudokephyrion ellipsoideum, P. obtusum and others), three  $\beta$ -mesosaprobic (Mallomonas acaroides, Synura peterseniii and Dinobryon divergens) and three  $\alpha$ -mesosaprobic species (Sycrypta pallida, S. volvox, S. xantha). Analysis of the values of the index of saprobity showed that the most water bodies where indicator organisms were identified (lakes, trout ponds, some watercourses) relate to the oligosaprobic zone or transitional oligo- $\beta$ -mesosaprobic subzone.

Floristic connections were established between the golden algae of the Mountainous Crimea, Ukrainian Carpathians and the Carpathians located abroad (the Polish, Romanian and Czech and Slovak Carpathians), indicating the unity of the mechanisms of florogenesis in the regions studied (fig. 2). Within the limits of the UC, according to physics-geographical zoning, the floras of Chrysophyta of Pre-Carpathians, Carpathians and Trans-Carpathians were compared. The Northern and the Southern macroslopes (NMS & SMS) of the Crimean Mountains main chain (Yayla) were chosen as objects for comparison of the regional floras (fig. 3).





Fig. 3 – The number of species common for regional floras of golden algae.

Comparative analysis of the Jacquard coefficient  $K_J$  values suggests that the predominance of similarity over the difference is more clearly seen for the floras Chrysophyta of the Ukrainian and foreign

Carpathians ( $K_J = 0.39$ ) than for those of the UC and the MC ( $K_J = 0.36$ ). It should also be recognized that the regional flora of the MC and the foreign Carpathians ( $K_J = 0.20$ ) even more different (fig. 4).



Fig. 4 – Maximum correlation paths, showing the degree of systematic structures similarity of the regional *Chrysophyta* floras to the values of the Kendel coefficient of rank correlation. Note:\* C – Carpathians, PC – Pre-Carpathians, TC – Trans-Carpathians, SM – Southern and NM – Northern macroslopes of Crimean Mountains

The lowest values of the Kendel coefficient were registered for pairs of Chrysophyta regional floras of the Carpathians and Trans-Carpathians (0.64), Southern MS and Carpathians (0.72), Carpathians and Pre-Carpathians (0.71). The maximum similarity in the processes of the phlorogenesis of golden algae was found in the Ukrainian and foreign Carpathian Mountains, because while comparing the head parts of the floristic spectra of families, the highest value of the Kendel coefficient ( $K_K = 0.82$ ) was obtained. Less flora of the UC and CM (0.78) are less close. The flora of the UC and MC are less phylogenetic close to each other (0.78). When comparing the systematic structures of the flora of chrysophytes MC and the foreign Carpathians, their insignificant similarities were established (0.69)that is apparently due to physics and geographic factors [7-9].

In our opinion, the position of the dynamic "weights" of similarity – difference between the compared floras is due to the result of a complex balance

of various factors of animate and inanimate nature that determine the dynamics of the qualitative and quantitative composition of each specific flora throughout phylogenesis.

Thus, on the one hand, flora of golden algae of the UC and MC are characterized by a high level of specificity of the species composition that is due to the large number of species individually represented in each flora, as well as the chronological isolation of florogenesis and the geographical distance of regional floras. In this case, the differences between the compared floras prevail over the similarity [4, 5].

On the other hand, the proximity of the study regions from an orographic point of view causes the formation of identical climatic conditions that means a similarity of environmental factors. The same conditions of existence, in turn, determine the similarity of the forms living in them, which, due to uniform adaptive reactions, acquire equal ranges of tolerance with respect to these abiotic and biotic environmental factors. This probably causes the systematic homogeneity of organisms (for example, golden algae) of comparative floras that is primarily occurs at the level of higher-ranking taxa.

Summing up, it should be noted that the regional algoflora of golden algae on the ecological and floristic "weights", demonstrate a pronounced predominance of similarity over the difference, which also evidence by statistical data obtained using different coefficients of floristic genuslity and rank correlation.

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The systematic structure of regional floras of Chrysophyta has a number of similar features and is characterized by a pronounced predominance of representatives of two orders of Chromulinales and Ochromonadales (94 %), or families Synuraceae, Dinobryonaceae, Chrysococcacea (80.5 %). A certain similarity of the systematic structure of golden algae in the studied physical and geographical regions appears at the level of the eight dominant genus Synura, Dinobryon, Chromulina, Kephyrion, Chrysococcus, Ochromonas, Mallomonas and Pseudokephyrion. The presence of a large number of species individually represented in the floras (60.6 % for CM and 55.9 % for UC) indicates a high level of specificity of their species composition.

The distribution of golden algae in water bodies of different types is uneven. The number of species of this algae's group decreases in the following sequence: ponds and reservoirs (70) - lakes (27) - rivers and streams (15) for UC and bogs (66) - lakes (55) - ponds and reservoirs (52) - rivers and streams (25) - for the CM. The most characteristic is the sequence of reducing the species richness of Chrysophyta in water reservoirs of the Ukrainian Carpathians. Each type of reservoir is characterized by a peculiar complex of golden algae species, however, a significant part of the species (30.5 %) is found in all types of reservoirs.

The distribution and spreading of the Chrysophyta group representatives in the areas studied is not even. A significant part of the identified species is narrowly localized, including 25.3 % which were revealed only in the waters of the Ukrainian Carpathians, and 17.8 % – in the Crimean Mountains. One fifth of the species (20 %) occurs throughout the study regions. Among them, mainly representatives of the genus Dinobryon and Chrysococcus ( $K_f = 4.79$ , and 6.62, respectively).

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