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THE COMPLEX STUDYING OF ANTARCTIC BIOTA

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Abstract. Results of five year period of Argentina islands region Antarctic biota complex investigations are described. There were described 41 algae new for the Galindez island biogeografical polygon territory. Check-list of terrestrial algae now consists of 57 species belongs to 3 phyla. 12 species from Chlorophyta and Bacillariophyta were described for snow and cryophilic communities from Uruguay and Galindez islands. The preliminary analysis of different chord animals' groups' phenological peculiarities were carry-out. 9 fish, 14 birds and 8 mammals' species were analyzed.Complex phenological investigations of terrestrial animals from bryophytic and soil substrata were carry-out for the first time. Virus antigens were detected in samples of *Deschampcia antarctica* and bryophytes from *Barbilophozia* and *Polytrichum* genera. This viruses belongs to different taxonomical groups such as Tobacco mosaic virus (*Tobamovirus*), Cucumber green mottle mosaic virus (*Tobamovirus*), Cucumber mosaic virus (*Bromoviridae, Cucumovirus*), Tomato spotted wilt virus *(Bunyaviridae, Tospovirus). Deschampsia antarctica* photosynthetic tissues samples investigations revealed high level of antioxidant systems on example of superoxide dismutase. Such activity shows *Deschampsia antarctica* high plasticity. Such peculiar properties of photosynthetic membranes of lipid-protein-pigment complexes structural and functional components are demonstration of active adaptive strategies to Antarctic limitative factors. Identified similarities lipid metabolism of various species may be indicative of the existence of a common ancestral form, but some differences in levels of lipid metabolism - on the next stages of evolutionary divergence in relation to the formation of a unique set of adaptations to the harsh Antarctic conditions. These data, together with the data of morphological, anatomical, physiological, molecular studies may be useful in assessing the evolutionary relationship of various species of Antarctic fish.
Key words: biocenosis, phenology, soil algae, plant viruses, adaptive strategies, lipid metabolism

Реферат. Стаття присвячена результатам п'ятирічного комплексного вивчення антарктичної біоти в районі Аргентинських островів. У результаті проведених досліджень систематичний список наземних водоростей біогеографічного полігону на острові Галіндез поповнився на 41 таксон видового рангу і включає 57 видів з трьох відділів. У групуваннях снігу та льоду на острові Галіндез та острова Уругвай виявлено 12 видів водоростей з двох відділів: Chlorophyta та Bacillariophyta. Проведено первинний аналіз фенологічних особливостей різних груп хордових тварин: 9 видів риб, 14 видів птахів і 8 видів ссавців. Уперше проведено комплексне фенологічне дослідження ґрунтових тварин із моху, грунт-субстрату та грунту. У зразках рослин *Deschampcia antarctica* та мохах родів *Barbilophozia* та *Polytrichum* були детектовані антигени вірусів, які належать до різних таксономічних груп, а саме вірус тютюнової мозаїки *(Tobamovirus)*, вірус зеленої крапчатої мозаїки огірка *(Tobamovirus),* вірус огіркової мозаїки (*Bromoviridae, Cucumovirus*), вірус плямистого зів'янення томатів *(Bunyaviridae, Tospovirus).* Дослідження фотосинтетичних тканин зразків *Deschampsia antarctica* засвідчують високий рівень антиоксидантних систем (на прикладі супер-оксиддисмутази), що говорить про достатньо високу пластичність (більша норма реакції) рослин виду *Deschampsia antarctica.* Виявлені особливості структурно-функціональних компонентів ліпід-білково-пігментного комплексу фотосинтетичних мембран *Deschampsia antarctica* є проявом активних адаптивних стратегій даного виду рослин до лімітуючих факторів Антарктики. Виявлені подібності ліпідного обміну різних видів риб можуть свідчити про наявність у них спільної предкової форми, а наявність відмінностей у деяких ланках ліпідного метаболізму – про наступні етапи еволюційної дивергенції у зв'язку з формуванням

унікального набору адаптацій до суворих умов Антарктики. Одержані дані наряду із даними морфологічних, анатомо-фізіологічних, молекулярних досліджень можуть бути корисними при оцінці еволюційної спорідненості різних видів антарктичних риб.
Ключові слова: біоценоз, наземні водорості, фенологія, віруси рослин, адаптивні стратегії, ліпідний метаболізм.

Реферат. Статья посвящена результатам пятилетнего комплексного изучения антарктической биоты в районе Аргентинских островов. В результате проведенных исследований систематический список наземных водорослей биогеографического полигона на острове Галиндез пополнился на 41 таксон видового ранга и включает 57 видов из трёх отделов. В группировках снега и льда на острове Галиндез и острова Уругвай выявлено 12 видов водорослей из двух отделов: *Chlorophyta* и *Bacillariophyta*. Проведен первичный анализ фенологических особенностей различных групп хордовых животных: 9 видов рыб, 14 видов птиц и 8 видов млекопитающих Впервые проведено комплексные фенологические исследования почвенных животных из мха, грунт-субстрата и грунта. В образцах растений *Deschampcia antarctica* и мхах родов *Barbilophozia* и *Polytrichum* были детектированы антигены вирусов, принадлежащих к разным таксономических группам, а именно вирус табачной мозаики (*Tobamovirus)*, вирус зеленой крапчатый мозаики огурца (*Tobamovirus*), вирус огуречной мозаики (*Bromoviridae, Cucumovirus*), вирус пятнистого увядания томатов (*Bunyaviridae, Tospovirus*). Исследование фотосинтетических тканей образцов *Deschampsia antarctica* подтверждают высокий уровень антиоксидантных систем (на примере супероксиддисмутазы), что свидетельствует о достаточно высокой пластичности (большая норма реакции) растений вида *Deschampsia antarctica*. Выявлены особенности структурно-функциональных компонентов липид-белково-пигментного комплекса фото-синтетических мембран *Deschampsia antarctica*, что очевидно является проявлением активных адаптивных стратегий данного вида растений к лимитирующим факторам Антарктики. Выявленные сходства липидного обмена различных видов рыб могут свидетельствовать о наличии у них общей предковой формы, а различия в некоторых звеньях липидного метаболизма – о следующих этапах эволюционной дивергенции в связи с формированием уникального набора адаптаций к суровым условиям Антарктики. Полученные данные наряду с данными морфологических, анатомо-физиологических, молекулярных исследований могут быть полезны при оценке эволюционной родства различных видов антарктических рыб.
Ключевые слова: биоценоз, наземные водоросли, фенология, вирусы растений, адаптивные стратегии, липидный метаболизм.

Introduction. Due to its climatic and ecological characteristics, the region of Antarctica is unique area of wilderness with unprecedented biodiversity. Hence complex study and continuous monitoring are the priority tasks when conducting research of such reference areas of untouched nature as Antarctica. Complex study of biodiversity presumes analyzing all inclusive elements: complexes of viruses, microorganisms, botanical and zoological objects. One of these are zoocenoses belonging to various ecosystems. Here animals utilize primary production or are predators – being the consumers of the first and higher orders. Thus, the ground substrate (by the example of Argentina Islands) for biota expansion are the rocks, where lichens grow, and crushed eluvium. Mosses dominate in vegetation, however two species of higher vascular plants, *Deschampsia antarctica* and *Colobanthus quitensis*, also grow on confined areas. Due to the extinction of vegetation and wind drift the areas of primary soil are formed. These are especially evident close to the rookeries, where significant accumulations of organic guano are formed. The representatives of multiple groups of invertebrates (free-living soil nematodes, tardigrades, mites, colembolas, chironomids, etc.) feeding on plants or preying on other invertebrate animals of these ecosystems are typical inhabitants of moss, soil substrate, lichen and *D.antarctica* sods. Therefore, because of the life activity of higher chordates (birds and mammals) the conditions are being created for producers' growth, primary production of whose is then utilized by the invertebrates enabling the transmission of power by the food chain. This is preconditions the necessity for complex study of such ecosystems, both for separate elements of zoo chain and for the biodiversity as the whole.

In addition, the Antarctica is the indicative example of global climate changes which, in turn, directly effect the alterations in biotic part of its ecosystems.

As of today, many aspects of existence of different groups of antarctic animals, but more importantly – many aspects of alterations invoked by direct of indirect human activity are studied insufficiently.

Plants, mosses and algae. It is known that the producers of terrestrial antarctic ecosystems are represented by algae (free-living ones and lichens' photobionts), moss-like (mosses and liverworts) and vascular plants. In terrestrial biotopes in the region of Argentina Islands we have identified: 78 species and intraspecies taxons of algae, 34 moss species, 2 species of higher plants (Deschampsia antarctica та Colobanthus quitensis) which altogether makes 114 species and intraspecies taxons of the producers.

The algae compose four types and 8 variants of grouping:

1. Litophilous (epilithic, endolithic, hypolithic)
2. Edaphic (of litosoil, ornithogenous soil, true brown soil)
3. Epiphyte groupings of bryocenoses
4. Cryophylic (groupings on snow and ice).

Following the inventory analysis of flora research for terrestrial algae of Argentina Islands, we have registered the following in the terrestrial phytocenoses of Antarctica: 267 species (2, 14), from which 150 species are found in coastal Antarctica.

In the region of Argentina Islands 78 species and intraspecies taxons were identified (10) (about 30% from the total number of terrestrial algae species in Antarctica). From these, 7 species belong to *Cyanoprokaryota*, 41 species – to diatomic algae (*Bacillariophyta*), and 30 species – to green algae (Chlorophyta).

In addition, we have identified 3 species which are completely new for Antarctica as the whole:

Komvophoron groenlandicum (Cyanoprokaryota) – rare species, earlier known from Greenland only. This species has been detected in litosoils from Galindez Island.

Pseudococcomyxa subellipsoidea (Chlorophyta) – one of the most spread species of epibryophytic and soil groupings in the region of Argentina Islands.

Elliptochloris bilobata (Chlorophyta) – a rare species for Antarctica belonging to epibryophytic groupings. Known mostly in mountain regions of Northern hemisphere. We have identified 6 taxons, whose morphotypes totally don't correspond to any diagnosis of any species described so far, hence these are new for science.

Four species are the genera which have not been identified in Antarctic terrestrial biotopes until now (Spirulina sp., Avernensia sp., Parietochloris sp.1, Parietochloris sp.2), when two new species belong to the genera which have been previously described in Antarctica (Coleochlamys sp. And Koliella sp.).

We have identified complexes of dominants for edaphic, epibryophytic and cryophilic groupings in the region of Argentina Islands: soil algae (57 species), cryophilic algae (12 species) and epibryophilic algae (39 species).

Following the outcomes of the inventory analysis for moss-like plants on Argentina Islands, 34 moss-like species have been detected, from which 29 moss species and 5 liverwort species (20).

Terrestrial ecosystems. The investigation of the invertebrates in terrestrial ecosystems (26) has been carried out for establishing species diversity for these animals in the region of Argentina Islands and also for studying changes in their phenology under extreme conditions of the changing environment.

The representatives of two [earlier] problematic animal groups (colembolas and mites) have been identified in some samples collected in 2007-2008 (22). Thus, the colembolas are represented by 3 species (*Cryptopygus antarctica*, *Friesea grisea* and *Isotoma octooculata*), when mites – by 5 species (*Alaskozetes antarcticus*, *Gamasellus racovitzai*, *Protereunetes minutus*, *Stereotydus villosus*, *Ixodes uriae*). Such complex data (many sampling points, large territory, seasonal sampling, and

cultivation) for different points from the large territory close to UAS 'Academician Vernadsky' has been generated for the first time in the history of Ukrainian research in Antarctica and for this geographical region on the whole. Moreover, all points have been already tested for chironomids with the single species identified as *Belgica antarctica*.

Fresh water ecosystems. In big freshwater pools gill-footed crustaceans were detected, *Brachinecta granulosa* for instance (Galindez, Skua, Barkhany Islands, etc.). Most importantly, during the expedition in 2007-2008, a copepod *Pseudoboeckella poppei* (6) have been found in the single small fresh water pond at the boundary island of the Rock group close to UAS 'Academician Vernadsky' which then has been identified in office conditions.

Fish. We have carried out analysis of phenology and morphometric characteristics for 9 fish species (12). For the first time, morphometric analysis has been completed for nearly 500 fish specimens providing the opportunity (if further research and sampling from other points permit) to assess morphological adaptations of fish in view of changing influence of the environment. As of now the conducted analysis shows that the subdominant has changed (for the first time during the years of monitoring) which is possibly due to the gradual change in climatic conditions. Thus, by quantity the notothenia *N.coriiceps* remains the absolute dominant in captures (72,4%), when marbled notothenia *N. rosii* became the subdominant (12%). Interestingly, only one specimen of marbled notothenia has been captured in previous years, when 57 – in 2007-2008. At the same time, mottled trematomus *Tr.bernacchii*, known to be always a subdominant in previous years, in 2007-2008 has been captured for only 4 times and once it has been found in the stomach of a predator fish which makes only 1.1% of its total quantity.

Birds. The monitoring of avifauna in the region of Argentina Archipelago (23) and further analysis of obtained results provided important information on phenology for 14 bird species. This monitoring has been started at the time of first Ukrainian expeditions to Antarctica and hence our investigations were in fact the continuation of long-term monitoring. Phonological research for birds and mammals allows noting certain deviations and establishing the underlying reasons. For instance, for the first time Jintu penguins founded new colonies on Galindez Island and Tucsen Cape where baby birds were raised. This is of great significance as at the present this is the first registered case of new southernmost nesting site of Jintu penguins in this region Antarctica. In our opinion, this is the global climatic changes of our planet that causes the reformation of the living environment for these birds.

Mammals. The above said tendencies of studying phonological characteristics are also peculiar to the mammals, 8 species of which have been registered. Thus, sea bear colonies in the region of UAS 'Academician Vernadsky' do gradually increase each year which is evident of more optimal conditions being created for the representatives of this species because of the changes in the living environment.

Viruses. Molecular biological analysis of total RNA preparations from *Deschampsia antarctica* and *Colobanthus quitensis* via RT-PCR for coat protein gene of TMV, PVX and CGMMV. Samples analyzed in RT-PCR have then been subjected to ELISA with antisera to CMV, CGMMV, TSWV, TMV, AMV, WSMV, BMV, BYDV, TuMV, PVX and PVY; this resulted in identification of AMV and CGMMV in plant sap (13).

Despite we chosen the optimal technique for isolation of total RNA from *Deschampsia antarctica* and *Colobanthus quitensis* plants, RT-PCR gained negative results only (3).
In view of this, the samples were further checked using ELISA for CMV, CGMMV, TSWV, TMV,

AMV, WSMV, BMV, BYDV, TuMV, PVX and PVY (Fig.). We assumed the presence of virus antigens for AMV and CGMMV in several samples of D. Antarctica collected at Galindez, Durbox, Lippmann Islands and Cape Rassmunssen, as colorimetric data have been close to positive values. Obtained results may be explained by very low antigen content, presence of closely related strains or temperature regimes during sampling which occurred at unfavourable conditions. This allows concluding on positive ELISA outcomes for some samples of *Deschampcia* and *Colobanthus* plants.

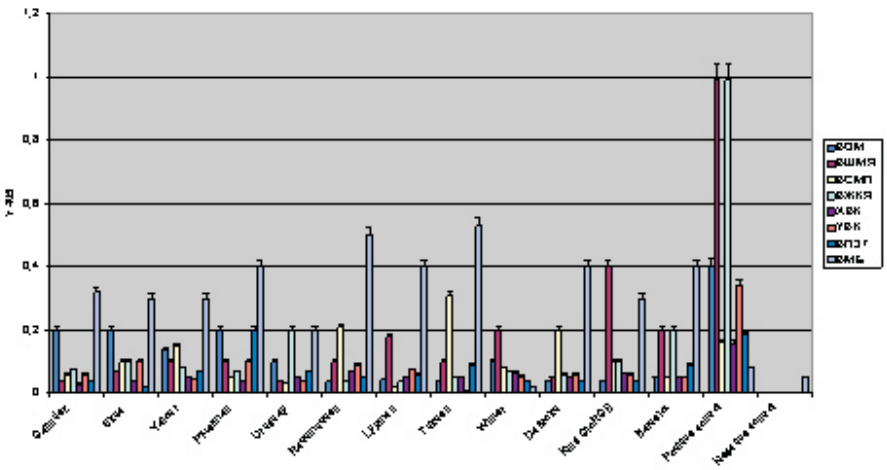

Figure. Virus detection in *Deschampcia antarctica* plants via DAS-ELISA.

Plant physiology. Investigation of adaptation of higher vascular plants (*D. antarctica* and *C. quitensis*) of Antarctica to abiotic factors of this region allowed to verify the presence of specific strategies of customizing to them, i.e. formation of the shortest ontogenesis that timely matches the term of the Antarctic summer, and provides quick phases of ontogenesis (similarly to ephemorous plants) necessary for formations of vegetative mass and maturation of generative apparatus of plants .

Together with passive adaptation the plants of *D. antarctica* also use active ways of adaptation, which were examined by us at the level of light dependent processes and involved in its regulation components of hardly investigated lipid-pigment complex of photosynthetic membranes of plants. The precedence of both of photosynthetic pigments and specific lipid structures (glycolipids and sulphohinovozylglycerol) involved in stabilization of funktion of plants photo systems under extreme factors was showed (17, 19). Comparison was held in species aspect with aboriginal plants of *Deschampsia caespitosa* and under conditions of experimental oxide stress (2, 15).

Investigation of pigment-protein complexes of tylakoid membranes of plants of *D. antarctica* showed quantitative differences in general content of light harvesting complex 2 (LHK II) – its oligomeric ($LHCP^{1}$) and monomeric ($LHCP^{3}$) forms, content of chlorophyll in CPa zone that corresponds to pigment-protein complexes of close antenna. Results of global alignment and domain architecture showed that the fragment of amino acid sequence of protein fraction of *D. antarctica* PSII is highly similar to the sequences of *A. thaliana* and *O. sativa* that belong to the protein family PcbC, and as a result is a fragment of product of psbC family gene. It was also noted that in this fragment C-terminal part of sequence, which by the size corresponds to one or more exones, is absent (18).

Study of photosynthetic tissues of *Deschampsia antarctica* samples, which were selected in Antarctica, also prove high level of antioxidative systems (as an example, superoxidedismutase), that verifies pretty high plasticity (higher reaction norm) of plants *Deschampsia antarctica* (16).

Determined features of structural and functional components of lipid-protein-pigment complex of photosynthetic membranes of *Deschampsia antarctica* are display of active adaptive strategies of plants of this species to limiting factors of Antarctica (1).

Biochemistry. During lots of decades Antarctica has been a universal polygon for investigations of morphological, physiological and biochemical-molecular features of endemic flora and fauna. Antarctica is a kingdom of nototheniid fish, as about 75% of representatives of bottom fauna of Antarctica belong to superfamily Notothenioidae (4, 7).

The problem of biological monitoring is search of biochemical indicators – both for estimation of state of natural populations and for identifications of level of species varieties.

Under conditions of environment during long-termed evolutional process different species of the Antarctic fish obtained morphofunctional features of adaptive character, that gave the possibility to customize both separate biochemical reactions and functioning of the whole metabolic systems regarding severe environmental conditions (5). One of the most important organic substances in live organisms are lipids, which characterize in significant structural variety and wide spectrum of functional activity (11).

This caused the necessity of investigation (2007-2008) of characteristics of lipid metabolism in organism of different species of the Antarctic fish for estimation of homeostasis support ways under adaptation to low temperatures. The blood serum is one of the most important pools of lipid substances, thus quantitative indexes of lipid substances in blood serum are informative while estimation of general level of lipid metabolism.

As a result of carried out work for three species of Antarctic fish. T.bernacchii, crocodile icefish (Ch.aceratus) and N. Coriiceps differences in the content of main lipid blood components: triazyl-glyerides, phospholipids and cholesterol, are determined (9).

Thus, increased level of TG in blood serum of icefish comparing to other species, can certify the possibility of their utilization as additional source of energy (21).

Content of cholesterol that has structural and regulatory functions, for these species of fish is averagely 5,8±0,7 mmol/l. Hence according to literature data the content of cholesterol in representatives of temperate, and especially tropical latitudes, both in membranes and blood serum is comparatively higher. That is why one of the fish adaptation mechanisms to low temperatures is prevention of decrease of membranes fluidity by decreasing of cholesterol pool.

In blood serum of T.bernacchii decreased level of phospholipids, main component of cellular membranes (1,95±0,10 mmol/l) was determined, comparing to C.aceratus and N.coriiceps, for which this index is 2,77±0,16 and 2,64±0,11 mmol/l, respectively. The content of main phospholipids (phosphatydilcholine and phosphatydilamine) for C.aceratus and T.bernacchii is similar and is averagely 36 and 19% from total volume of phospholipids. Hence, for the representatives of species N.coriiceps the tendency is contrary (25 and 36%, respectively). The content of minor component of phospholipids (sphingomielin, phosphatydilserine and phosphatydilinozitol) in blood of all fish species doesn't differ significantly. Determined differences in the content of phospholipids and redistribution of individual phospholipids show the features of membrane dependent processes for different species of Antarctic fish (8, 24, 25).

Lipids of blood serum predominantly are the part of lipoproteins (LP), which main function lies in lipid transport between organs and regulation of lipid metabolism. Differences in content of different class LP and in indexes of correlation between different LP classes in blood serum of the Antarctic fish are determined. It shows the differences in metabolic cycles caused by lipid homeostasis.

Obtained results of studying of content of lipids and lipoproteins of different species of the Antarctic fish verify the presence of their features of energetic and metabolic processes connected to adaptation to conditions of Antarctic. Obtained data show that C.aceratus and T.bernacchii characterize by the similarity of indexes of lipid metabolism. At the same time N.coriiceps under such indexes demonstrates significant differences from these studied species. It is ought to note that N.coriiceps has a wider residence area (representatives can be met in the seas distal from Antarctica, and sometimes in temperate latitude waters) comparing to T.bernacchii and Ch.aceratus, which are

exclusively Antarctic. It can confirm that the presence of specific features of lipid metabolism of N.coriiceps is one of the factors causing a width of adaptive spectrum to different environmental conditions.

Identified similarities for the studied indexes of lipid metabolism of these species can witness about presence of common ancestor form, and presence of differences in some steps of lipid metabolism- about further stages of evolutional divergence due to formation of unique adaptation set to severe conditions of Antarctica. Obtained data, together with data of morphological, anatomic, physiological and molecular investigations can be useful while estimation of evolutional relationship of different species of the Antarctic fish.

Thus, as of June 2008, according to results of our investigations the systematic list of terrestrial algae from biogeographical polygon on island Galindez was amended by taxon species and included 57 species from 3 phylum Cyanophyta (4 species), Bacillariophyta (30), Chlorophyta (23).

12 algae species from two phylum Chlorophyta (11 species) and Bacillariophyta (1) were identified in samples of snow and ice taken from biogeographical polygon on Galindez island and from territory close to UAS 'Academician Vernadsky' Urygvay island.

Primary analysis of phenotypical features of different chordata animal groups: 9 fish species, 14 avian species and 8 mammalian species was conducted.

Complex phonological investigations (during one year period) of terrestrial invertebrates from moss, soil-substrate and soil were conducted for the first time.

We have detected antigens of viruses belonging to different taxonomy groups: Tobacco mosaic virus (*Tobamovirus*), Cucumber green mottle virus (*Bromoviridae, Cucumovirus*), Tomato spotted wilt virus (*Bunyaviridae, Tospovirus*) in samples of plants *Deschampcia antarctica* and moss of *Barbilophozia* and *Polytrichum genus.*

The results of our investigations showed unexpected high diversity of viral antigens detected in Antarctica.

Researches of samples from *Deschampsia antarctica* photosynthetic tissues proved high level of antioxidative systems (as an example, superoxidedismutase), that verifies pretty high plasticity (higher reaction norm) of plants *Deschampsia antarctica.*

Determined features of structural and functional components of lipid-protein-pigment complex of photosynthetic membranes of *Deschampsia antarctica* are display of active adaptive strategies of plants of this species to limiting factors of Antarctica.

Identified similarities for the studied indexes of lipid metabolism of these species can witness about presence of common ancestor form, and presence of differences in some steps of lipid metabolism- about further stages of evolutional divergence due to formation of unique adaptation set to severe conditions of Antarctica.

Obtained data, together with data of morphological, anatomic, physiological and molecular investigations can be useful while estimation of evolutional relationship of different species of the Antarctic fish.

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