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PHOTODYNAMIC THERAPY IN THE TREATMENT OF PATIENTS WITH PURULENT WOUNDS

Abstract. Nowadays, a relatively new medical technology, photodynamic therapy, is being actively developed and introduced into clinical practice. The method is based on the ability of many biological objects (tumor, microbial cells) to accumulate chemicals - photosensitizers, after which they become sensitive to sunlight, as well as to laser radiation of a certain wavelength. In cells that have absorbed a photosensitizer, a photochemical process is launched with the generation of singlet forms of oxygen, which has a destructive effect on biological systems. The review presents information on the use of photodynamic therapy, based on the integrated use of light and chemical compounds - photosensitizers, which is one of the areas of laser medicine. The relevance of the review of the existing capabilities of PDT is due to the great interest of surgeons in the use of this method in the treatment of purulent wounds. The article provides a brief historical background on the development of this method of treatment, covers the principles and mechanisms of its action. The possibilities of using photodynamic therapy in surgical practice are analyzed. The prospects of further development of the method for the treatment of purulent-necrotic wounds are substantiated.

Key words: laser medicine, photodynamic therapy, photosensitizer, purulent wound.

The treatment of purulent wounds of various etiologies is an urgent problem of surgery up to the present time and has a tendency to grow, has a long history [1]. One of the principles of treatment of purulent wounds of various origins is the use of antibacterial drugs of various groups that cannot guarantee reliable prevention of infectious complications, due to the rapid adaptation of wound microflora, as a result of which the formation of antibiotic-resistant microorganisms occurs. It is necessary to take into account the decrease in the immunological response of the body after the use of drugs in this group, a violation of intestinal flora [1-3]. Despite the success of surgery over the past decades, it should be noted that the frequency of purulent complications remains almost unchanged. For a long time, mankind has been searching for methods of treating wound infections, there have been promising achievements - this is largely due to the discovery and the beginning of the use of antibacterial agents in the first half of the 20th century, and subsequently with the use of proteolytic enzymes. However, these methods did not have a universal effect on the wound process, needing further development [1, 2]. To date, the principle of treatment of purulent wounds involves a wide disclosure of the suppurative focus, followed by open wound management [1-3]. The disadvantages inherent in this method are the impossibility of carrying out a frequent change of gauze dressings during the day, damage to the forming granulation tissue when changing the dressing [3]. This creates prerequisites for increasing the duration of treatment, creating economic losses for the patient and for the state due to the long disability time. The most important condition for the local treatment of wounds in the postoperative period is drainage, the task of which is to remove wound exudates and products of wound exudates, the application of modern wound coatings, such as sorption, protective, containing drugs, atraumatic is also important [3]. Treatment of purulent wounds should be complex, taking into account the clinical manifestations of the disease of a single patient, the

presence of background pathology, indications and contraindications for the appointment of surgical and medical treatment [3].

Photodynamic therapy (PDT) seems to be a promising method in the treatment of purulent wounds as part of complex treatment, which uses a combination of low-frequency laser radiation with a wavelength of 630 - 1300 nm in combination with photosensitizers, leading to the development of a photochemical reaction in the presence of interstitial oxygen, which has a destructive effect on intracellular structures [4]. PDT is a relatively new trend in the treatment of purulent wounds; by now, sufficient clinical experience has been gained in using this technology, which is reflected in many papers [5, 15]. The launch of a photodynamic reaction is possible in the presence of a special chemical - a photosensitizer and light. The main active substrate is the photosensitizer, the latter is absorbed by the target cell, the inactive photosensitizer is inert and has no effect [6]. Absorbed quantum of light of a certain wavelength can lead to the activation of a substance molecule [5, 18]. The light source must have the necessary power, which will allow to deliver the radiation energy to the target cell and the molecular oxygen present in it, which is in a stable state and is characterized by the lowest level of molecular energy [5, 22]. Under the action of a photosensitizer in the presence of light, the oxygen molecule passes into a singlet form, which has a high chemical activity, with subsequent damage to the cell structures. Damage to biological structures, necrotic and apoptotic changes are the result of the launch of free radical reactions. The oxidation of biologically important molecules under the influence of visible light in the presence of molecular oxygen and a photosensitizer is called the photodynamic effect [5].

Currently, PDT is actively used in many countries and has such advantages as low invasiveness, selectivity of effects on the pathological focus, the ability to repeat courses of PDT, low systemic toxicity. [7-10]. The method has advantages over antimicrobial treatment, since the effectiveness of PDT is not related to the spectrum of sensitivity of microorganisms to antibiotics, it is successful even in the treatment of antibiotic-resistant strains of Staphylococcus aureus, Escherichia coli and other microorganisms that do not develop resistance to PDT, unlike exposure to antibiotics. Tissue damage in PDT is local, the bactericidal effect is limited to the area of laser irradiation of photosensitized tissue, thus avoiding the side effect that occurs when using antibacterial drugs for the treatment of surgical infection with local PDT. The method has found widespread clinical application in oncology, due to the selective accumulation of the photosensitizer in tumor tissue. Activation of the drug occurs when a local light exposure to the wavelength corresponding to the maximum absorption, leading to the generation of singlet oxygen, which leads to damage to the intracellular structures of the tumor cell [12, 14, 17, 20].

Nowadays PDT has found extensive use in many fields of medicine, the method is used to treat tumors of the skin, breast, lungs, bladder, infectious diseases, certain diseases of the skin and eyes, ENT organs, oral cavity. The effectiveness of such treatment is high with minimal load on the body [11, 13, 22, 23].

To date, there are more than 400 substances that have a photosensitizing effect. Derivatives of hematoporphyrin, chlorine, 5-aminolevulenic acid, phthalocyanine are most common in the manufacture of medical drugs [16, 18, 19, 26].

The use of photochemical reaction energy for treatment has been known for over 6000 years, when light-sensitive substances of some plants were used to treat skin diseases, in particular, for the treatment of vitiligo, a powder of dried parsley leaves, St. John's wort, parsnip was used, which was applied to depigmented areas of the skin and insolated with sunlight before the appearance of pigmentation by the type of tan [5, 21]. For the treatment of the same pathology, Ammimajus plant powder (large Ammi or Chinese cumin) was later used, the phototherapeutic effect was due to the content of photocoumarins, which have a pronounced phototherapeutic effect, and in the twentieth century, Ammimajus was used to synthesize the medical drug Ammifurin, used to treat vitiligo, psoriasis, lichen planus, neurodermatitis [18, 21].

O. Raab discovered the photochemical oxygen-dependent reaction for the first time in 1897. As a student at the University of Munich Pharmacological Institute, he studied the effects of dyes on paramecium microorganisms (Paramecium) [21, 28, 32, 33]. He noticed that these microorganisms that are in a solution of acridine orange die when they hit the light, but when they are in the dark they can move freely [21, 26, 28]. Further studies conducted under the guidance of Professor H. Tappeiner suggested that fluorescent substrates like acridine dye transform light energy into an active chemical reaction that leads

to the death of microorganisms [21, 29, 31]. Based on the results of a study by H. Tappeiner and X. Jesionek, in 1903, the first PDT session was performed for a patient with skin cancer, using eosin as a photosensitizer [34]. In 1905, they described the results of treatment of 6 patients with basal cell carcinoma of the face skin by local application of 1% eosin solution and prolonged exposure to sunlight or artificial radiation of an arc lamp [21, 30]. They managed to achieve complete resorption of foci in 4 patients with a duration of 1 year without a recurrent period. At the same time, H.Tappeiner and A. Jodlbauer coined the term "photodynamic action" [35]. In 1908 W.H. Hausmann makes a report on the phototoxicity of hematoporphyrin and concludes that hematoporphyrin is an active sensitizer for paramecium and red blood cells [36].

For the first time in 1912 F. Meyer-Betz experimentally demonstrated the effect of hematoporphyrin on the human body on itself [37]. After intravenous administration of 0.2 g of hematoporphyrin, solar photosensitivity is observed, manifested as edema and hyperpigmentation, lasting up to 2 months [21, 37].

The development of photodynamic therapy was promoted by the discovery of a new photosensitizer, hematoporphyrin, which showed higher efficiency compared to previous analogues. After some time, a derivative of hematoporphyrin (HGP, Hematoporpherinederivate - HpD) was synthesized, which turned out to be 2 times more effective than the original compound, but its toxicity declined higher [5, 38]. This drug was obtained by S. Schwartz by acting hematoporphyrin with concentrated solutions of sulfuric and acetic acids, HpD was used in the USA in 1960 for the diagnosis of neoplastic diseases [39].

The use of lasers in medical practice contributed to the further development of the method (the first half of the 1960s), since the laser was monochromatic, using the most optimal wavelength for a particular photosensitizer, leading to a higher intensity of the photochemical reaction [26], it also became possible to transfer the light flux through fiber-optic systems with targeted impact on the organs and tissues of the body containing a photosensitizer, which certainly led to the intensive use of the method in various fields of clinical practice [5, 26].

In PDT with photosensitizers of the first generation, positive results were obtained in the treatment of patients with purulent wounds, acceleration of wound cleansing from purulent necrotic detritus, stimulation of tissue regeneration processes was noted. At the same time, the use of photochemical preparations of the first generation was also marked by significant shortcomings, such as a long half-life from the body, often enough allergic reactions and a significant increase in the photosensitivity of the whole body for a long period of time [23].

The drug Photofrin II is used most often today, it is called the "workhorse" of PDT [21], cumulated in all tissues and organs of the reticuloendothelial system [16]. The longer delay of Photofrin II is noted in the tumor tissue, however, the prolonged delay in the skin cells (even at minimum concentration) of the photosensitizer dictates the need to limit the light regimen by patients for 4-6 weeks, to prevent skin burn like sunburn [5, 10, 21]. In Russia, the analogue of Photofrin II is a drug Photogem, synthesized under the guidance of Professor A.F. Mironov in 1990. Photohem is fluorescent in the red region of the spectrum, which also allows it to be used to verify the tumor process of determining its boundaries [5, 7, 21].

In the mid-1990s, clinical trials of a second-generation photosensitizer, Photosens, began in Russia. The drug has an intense absorption band in the red region of the spectrum 665-675 nm. High photochemical activity in the red region of the spectrum, higher transparency of the tissue for laser radiation, which allows to affect deeper tissues are the advantages of second-generation photosensitizers over the first, since the main limitation of the PDT method is the depth of penetration of laser radiation into the tissue [5, 21]. From the point of view of the methodology, the development of algorithms for the individual selection of parameters of light exposure in PDT seems promising. For example, of great interest is the possibility of selecting the density and dose rate of laser irradiation based on the data of fluorescent diagnostics [40].

The parameters to which the optimal photosensitizer must correspond, including biological, photophysical and chemical-technological criteria, were determined as a result of years of research. They are low toxicity, high elimination, high absorption in the spectral range, high selectivity of drug absorption by tumor cells [5, 7, 20]. The creation and introduction of photosensitizers with the ability to accumulate at a high rate in the tumor tissue and to quickly decay is of particular interest [5, 24]. One of the most significant factors limiting the possibilities of the method is the depth of penetration of the laser flux [5, 7].

Contraindications to laser PDT are the presence of malignant neoplasms, decompensation of cardiovascular activity, acute impairment of the cerebral circulation, hepatic and renal failure [41].

In the study of professors A.V. Geinitz, P.I. Tolstykh, V.A. Derbenyov et al. demonstrate a positive clinical effect of PDT in 80 patients with purulent wounds of soft tissues, the majority of patients were operated on with phlegmon and abscesses of various localization. The application of gel "Photoditazine" 0.1% on the wound was carried out, the exposure of the drug on the wound was not less than 2 hours. When exposed to PDT, researchers recorded an acceleration in the rate of wound cleansing from necrotic masses (within 2-3 days), allergic reaction to the photosensitizer was not registered. Evaluation of the results of treatment of patients with nonhealing wounds shows faster cleansing of the wound from necrotic masses, early appearance of granulation and marginal epithelization in patients of the main group, which was carried out photodynamic therapy, a decrease in the microbial contamination of wounds was recorded, this is due to the high level of accumulation of exogenous photosensitizer by microbial cells, and their own cells located in the area of the wound defect accumulate photosensitizer in a much smaller volume compared to bacteria. When studying local microcirculation, a decrease in edema in the area of a nonhealing wound, improvement of blood flow in the capillary bed, formation of a microcirculatory network, reduction of vascular resistance were noted. The expressed bactericidal effect of PDT on the basis of cytological data, rapid wound cleansing (3-5 days for the first or second session of photochemotherapy) was shown. Activation of the macrophage reaction leads to the stimulation of the wound process. After 1-2 weeks, the number of macrophages in the tissue increases significantly, mature macrophages with active phagocytic function prevail. In the main group, a more rapid transition of the wound process from the inflammatory to the reparative phase, maturation by the end of the second week of full-fledged granulation tissue, the transformation of the latter by the end of the third week into fibro-cicatricial, epidermis regeneration takes place, the wound size decreases due to epithelialization and contraction of the scar tissue. PDT in the treatment of nonhealing wounds contributes to the acceleration of the torpid wound process consistently leading to a reduction in the time of all phases of wound or ulcer healing. The method developed by the authors showed high efficacy compared with traditional treatment, allows to reduce the time of epithelialization of the wound 1.7 times while providing a good functional and cosmetic effect [25].

The advantages of antibacterial PDT include the same efficacy in acute and chronic infection, the method is also effective against bacteria, protozoa, fungi and viruses, photosensitizer does not have toxic and mutagenic effects; PDT does not depend on the spectrum of sensitivity of microorganisms to antibiotics; PDT's bactericidal effect is local, limited to the laser irradiation zone of sensitized tissues, which avoids microflora damage typical for antibiotics in areas not subject to irradiation, the possibility of repeated courses of treatment and combination in one diagnostic and treatment procedure [26, 27]. An additional advantage of PDT is its relative painlessness and the possibility of repetition in an outpatient basis.

As for the efficacy of PDT in patients with purulent-necrotic wounds, V.S. Panteleev in his study [42], demonstrated the effectiveness of skin graft engraftment by photodynamic effect “Photoditazine” in combination with laser antibiotic therapy. The authors provide information that as a result of the treatment, namely: patients with purulent necrotic wounds in the first study group, the removal of necrotic masses were removed using a low-frequency ultrasonic cavitator "SONOCA – 180" (Germany). In the second main group necrectomy was performed using carbon dioxide surgical laser "Lancet" (Russia), the third group was the control group. After the necrectomy stage, all patients of both main groups were subjected to a photodynamic effect with the second generation of photosensitizer “Photoditazine” in the form of 0.5% penetrator gel at the rate of 1 ml of gel per 45 cm^2 of the irradiated surface. After 2 hours from the moment of applying the FS, laser irradiation of the wound was performed using the Atkus-2 laser apparatus (Russia) in continuous mode with a power density of 1 W/cm^2 and a wavelength of 661 nm. In both major groups, antibiotics were activated by intravenous laser irradiation of blood (ILBL). As a result of the technique, the authors managed to reduce the time of preparing the wound surface for autodermoplasty by an average of 3 days, reduce antibiotic therapy by 1.3 times, and improve the effectiveness of skin graft engraftment by 24%.

Prof. Tolstykh P. I., Derbenev V. A. et al. [43] demonstrate the results of treatment of 129 patients with purulent wounds of different localization on the background of the use of a photosensitizer of the

chlorin series (photoditazine) in patients of the main group, the reduction of the time required for cleansing wounds and the earlier beginning of epithelialization in patients of the main group was shown on the background of a more rapid decrease in bacterial contamination of the wound. A morphological study proved more rapid relief of inflammation, reduction of microcirculatory disorders, increased phagocytic activity of neutrophils, accelerated maturation of granulation tissue during photodynamic therapy, and an elastic scar was formed in the main group of patients in a shorter time.

As a result of the research, the list of diseases, including surgical profile, for the treatment of which photodynamic therapy can be used, is constantly expanding. The possibilities of using this method are constantly expanding and photodynamic therapy is an alternative to the already existing methods and approaches in the treatment of purulent surgical diseases.

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ІРІҢДІ ЖАРАМЕН ПАЦИЕНТТЕРДІ ЕМДЕУ КЕЗІНДЕГІ ФОТОДИНАМИКАЛЫҚ ТЕРАПИЯ

Аннотация. Қазіргі уақытта жаңа медициналық технология – фотодинамикалық терапия клиникалық практикаға белсенді түрде дамып, енгізілуде. Әдіс көптеген биологиялық объектілердің (ісік, микробтық жасушалар) химиялық заттарды – фотосенсибилизаторларды кумулизациялау қабілетіне негізделген, содан кейін олар күн сәулесіне, сондай-ақ толқынның белгілі бір ұзындығының лазерлік сәулеленуіне сезімтал болады. Фотосенсибилизатор сіңдірілген жасушаларда биологиялық жүйелерге деструктивті әсер ететін оттегінің синглетті формаларының генерациясымен бірге фотохимиялық процесс іске қосылады. Шолуда лазерлік медицинаның бір бағыты болып табылатын жарықты және химиялық қосылыстарды – фотосенсибилизаторларды кешенді қолдануға негізделген фотодинамикалық терапияны қолдану мәселелері бойынша мәліметтер берілген. ФДТ-ның қазіргі мүмкіндіктерін шолудың өзектілігі іріңді жараларды емдеуде осы әдісті қолдануға хирург мамандардың үлкен қызығушылығын тудырды. Мақалада емнің осы әдісін әзірлеу туралы қысқаша тарихи оң жағында келтірілген, оның әрекет ету принциптері мен механизмдері көрсетілген. Хирургиялық тәжірибеде фотодинамикалық терапияны қолдану мүмкіндіктері талданады. Іріңді-некрозды жараларды емдеу үшін әдісті одан әрі әзірлеудің перспективасы негізделеді.

Түйін сөздер: лазерлік медицина, фотодинамикалық терапия, фотосенсибилизатор, фотодинамикалық терапия, іріңді жара.

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ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ ПРИ ЛЕЧЕНИИ ПАЦИЕНТОВ С ГНОЙНЫМИ РАНАМИ

Аннотация. В наши дни активно развивается и внедряется в клиническую практику относительно новая медицинская технология – фотодинамическая терапия. Метод основан на способности многих биологических объектов (опухолевые, микробные клетки) кумулировать химические вещества – фотосенсибилизаторы, после чего они становятся чувствительными к солнечному свету, а также лазерному излучению определённой длины волны. В клетках, которые абсорбировали фотосенсибилизатор запускается фотохимический процесс с генерацией синглетных форм кислорода, который обладает деструктивным влиянием на биологические системы. В обзоре представлены сведения по вопросам применения фотодинамической терапии, основанном на комплексном применении света и химических соединений – фотосенсибилизаторов,

являющимся одним из направлений лазерной медицины. Актуальность обзора существующих возможностей ФДТ обусловлена большим интересом специалистов-хирургов к применению данного метода при лечении гнойных ран. В статье приведена краткая историческая справа о разработке данного способа лечения, освещены принципы и механизмы его действия. Анализируются возможности применения фотодинамической терапии в хирургической практике. Обосновывается перспективность дальнейшей разработки метода для лечения гнойно-некротических ран.

Ключевые слова: лазерная медицина, фотодинамическая терапия, фотосенсибилизатор, фотодинамическая терапия, гнойная рана.

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INTRACORPOREAL RESECTION OF THE KIDNEY IN COLD ISCHEMIA WITH REGIONAL PERFUSION

Abstract. Renal cell carcinoma (RCC) is one of the most important problems of oncourology, due to the annually increasing morbidity and high mortality rate. According to the cancer registry, the incidence of renal cell cancer in the Republic of Kazakhstan (RK) occupies 12-13 rank places in the frequency of occurrence among all oncopathologies, on average, equally often affecting both sexes.

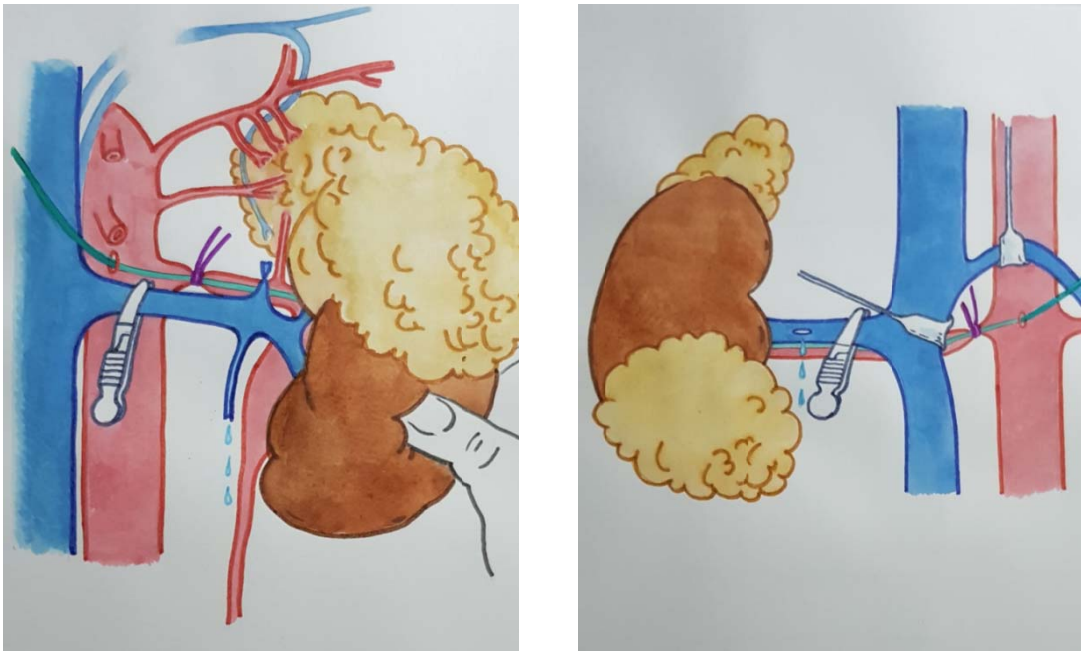
Keywords: carcinoma, oncourology, renal, cell.

Renal cell carcinoma (RCC) is one of the most important problems of oncourology, due to the annually increasing morbidity and high mortality rate. According to the cancer registry, the incidence of renal cell cancer in the Republic of Kazakhstan (RK) occupies 12-13 rank places in the frequency of occurrence among all oncopathologies, on average, equally often affecting both sexes [1]. Among the malignant neoplasms of the genitourinary system in the RK, RCC ranks 2nd after prostate cancer [1]. As is known, the main method of treatment of RCC is surgical and until recently the standard of treatment was radical nephrectomy. However, in recent years, with the improvement of diagnosis, early stages of kidney cancer have begun to be detected and the advantage of organ-preserving methods of treatment has been proved. According to the latest protocols of diagnosis and treatment of RCC, tumors of category T1a (up to 4 cm) are subject to partial nephrectomy (ie, resection of the kidney), and in tumors of category T1b (4 to 7 cm) - should be an individual approach based on the experience of the surgeon, the possibilities of the clinic and localization of the process in the kidney. This, as a rule, refers to the choice of resection methods for elective (electoral) indications. Quite a difficult situation arises with relative, and even more difficult with absolute indications, when it is necessary to decide on the feasibility and technically possible implementation of organ-sparing intervention in patients with cancer of the only / only functioning kidney with a large or centrally located formation, or with multifocal tumor growth. Currently, in this group of patients, the treatment strategy is reduced to nephrectomy with the introduction of the patient into the renoprival state with subsequent hemodialysis, or extracorporeal resection of the kidney with its subsequent autotransplantation. And if nephrectomy followed by hemodialysis dramatically worsens the quality of life of patients, extracorporeal resection of the kidney has many disadvantages and is accompanied by a large number of complications (risk of damage to the renal vessels, the risk of rejection of autograft, volemic, metabolic and hypothermic complications). As can be seen from the above, this category of patients is extremely difficult, both in the choice of treatment tactics and subsequent management and requires hemodialysis machines or other methods of detoxification. All of the above requires careful selection of patients and the choice of optimal treatment tactics, the search for alternative therapies.

The aim of the study was to improve the results of organ-preserving treatment of the only / only functioning kidney.

Material and methods. This method of surgical treatment was used in 4 patients with cancer of the only or only functioning kidney: in 2 cases, the right and in 2 cases, the left kidney. After median laparotomy, complete mobilization of the kidney and infrarenal aorta was performed. After preliminary bolus

heparinization, renal artery cannulation was performed punctually through the aorta. Blood flow to the kidney was isolated by clamping an artery over the cannula and renal vein at the confluence of the IVC. Then there was the regional perfusion of chilled (5-8^0 C) Custodial saline through a cannula in the renal artery. To prevent the ingress of this solution into the systemic circulation, the gonadal vein was crossed on the left, the lumen of the renal vein was opened on the right, through which the perfusion fluid was evacuated and the complete washing of the kidney from the blood to the pure solution was carried out (picture 1 A and B). Additionally, the kidney was covered with ice outside. Then resection of the kidney was carried out with the removal of the tumor and followed by suturing the cups, vessels and parenchyma of the kidney. After the resection was completed, the defect in the venous wall was sutured, the cannula was removed, the kidney was connected to the systemic blood flow and the defect in the aorta was sutured.

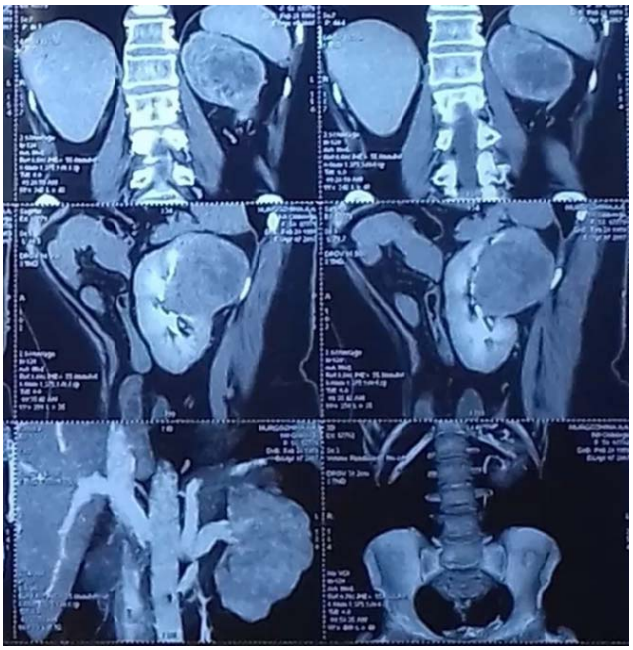


A B

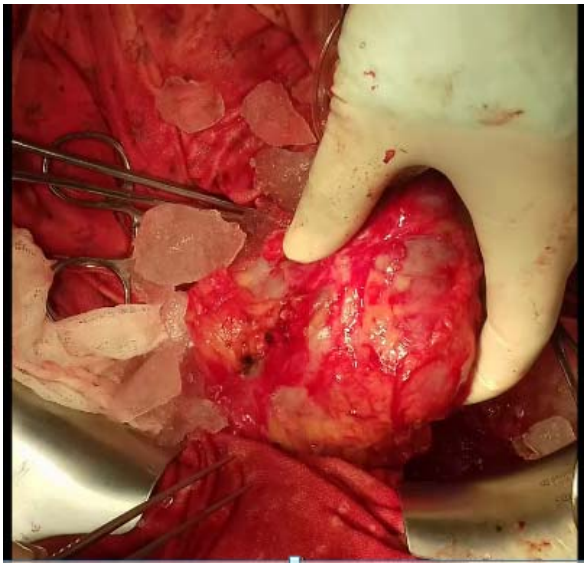
Picture 1 – a schematic view of kanalirovaniya renal artery (A – left, B – right) with irrigation of perfusion solution into the kidney and its isolation

Results. The average time of ischemia was 100 minutes (minimum 60 minutes, maximum 117 minutes). The average volume of blood loss is 345 ml (maximum – 500 ml). The maximum volume of formation is 10.5 cm. In three cases, a single tumor, in one - two tumors. In no case did not require additional appointment of extracorporeal detoxification methods. All patients before the operation, the value of creatinine was not greatly exaggerated, the highest level was in the range of 125 μmol/l. In the early postoperative period, the maximum value was in the range of 230 μmol/l, the rise was observed on 2-3 days after surgery. After the restoration of gastrointestinal function, there was a decrease in creatinine to preoperative levels. In 3 cases, kidney cancer was detected, in one – angiomyolipoma. Among the three cases of kidney cancer during the follow-up period (2 years), remission was observed in 2 cases, in one – progression after 6 months with the appearance of liver metastases.

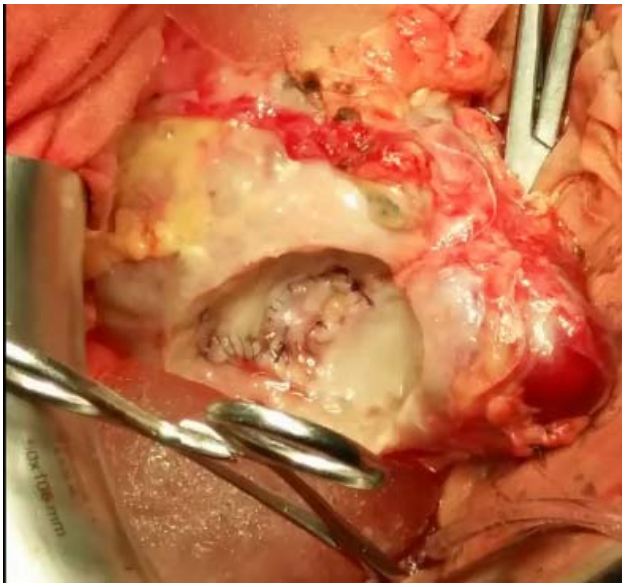
The following figures show a case study. Patient, N. 63 years old, Diagnosis: Carcinoma of the right kidney, condition after nephrectomy (2012), progression, metastasis to the only remaining left kidney the size of 9,5x6,0x5,0 cm, sprouting into the sinus of the kidney and the upper group of cups. Resection of the only remaining left kidney was performed according to the above procedure. The following pictures show the results of MSCT before surgery, intraoperative view, MSCT results in 2 years after surgery.



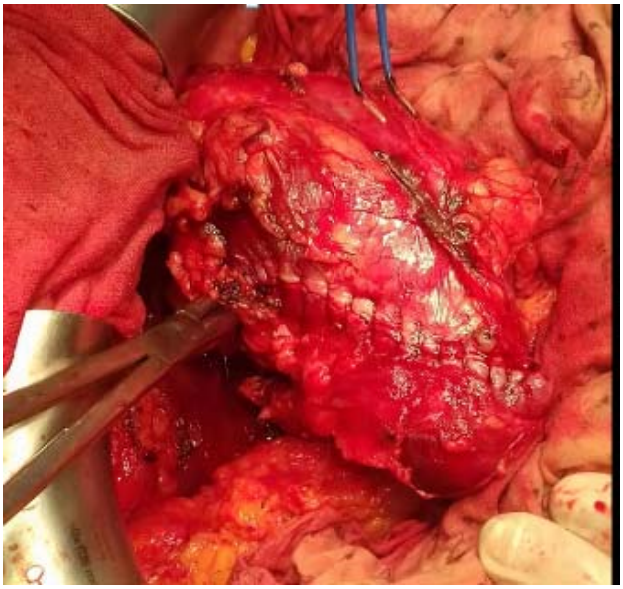
Picture 2 –
The results of the MSCT with bolus amplification carried out before surgery



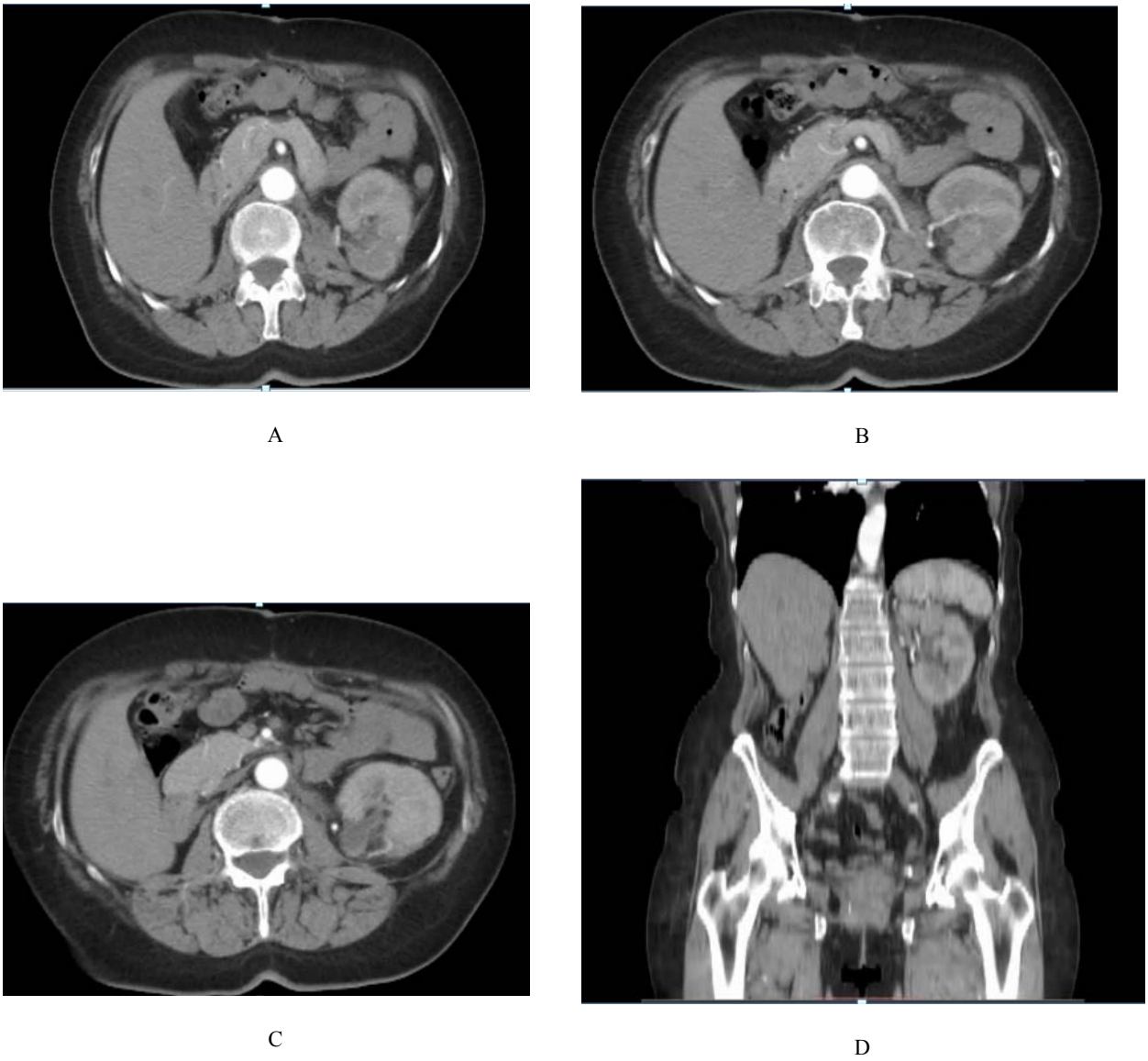
Picture 3 –
The left kidney is mobilized, the formation located in the upper pole is seen, mainly along the lateral edge with the transition to the back surface



Picture 4 –
View of kidney after resection and suturing of the abdominal system, the sinus of the kidney, blood vessels. Kidney of "white" color is a result of laundering it from formed elements



Picture 5 –
The final view of the kidney after resection – the kidney is connected to the systemic circulation



A B C D

Picture 6 (A,B,C,D)– the result of MSCT of abdominal and retroperitoneal organs in different scanning planes – without signs of recurrence (2 years after resection)

Discussion. This technique was developed by a team of authors on the basis of the Kazakh Research Institute of Oncology and Radiology, when we were forced to go for extracorporeal resection of the only remaining left kidney with a 9 cm tumor growing into the sinus and the upper cup. However, intraoperatively, we faced certain difficulties and had to change tactics, in addition, the lack of "artificial kidney" devices in the clinic increased the risk of possible complications. To increase the tolerance of renal tissue to ischemia, local hypothermia with icing on the kidney, perfusion therapy with cardioplegic solutions, which is used for extracorporeal resection and sometimes for resection in vivo, can be used. From the literature it is known that in the latter case, the perfusion of the renal vessels (artery) was performed by puncture with a syringe needle or opening of the lumen of the artery with subsequent irrigation solution [2,3]. Our technique was characterized by the fact that we cannulated the renal artery through the aorta, which is a great advantage due to the fact that the intima is not damaged and does not develop in the subsequent narrowing of the renal artery. And the second, irrigation is carried out with the necessary volume and creates sufficient pressure in the vessels and capillaries of the kidney, contributing to adequate washing them from the blood elements. It turned out that a similar technique has already been carried out by colleagues from St. Petersburg, which was published in the journal «Урологические

ведомости» (2015, №1) [4]. This technique was used to treat cancer of the left kidney, but the article States that in such situations on the right side they performed an autopsy of the renal artery with its cannulation, which in our opinion is impractical.

Conclusion. Thus, this method of operation has obvious advantages, low risk of postoperative complications, good results with careful selection of patients and in some situations can serve as an alternative to extracorporeal resection of the kidney.

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РЕГИОНАРЛЫҚ ПЕРФУЗИЯСЫ МЕН БҮЙРЕКТІҢ СУЫҚ ИШЕМИЯ КЕЗІНДЕГІ ИНТРАКОРПОРАЛДЫҚ РЕЗЕКЦИЯСЫ

Аннотация. Бүйрек жасушаларының карциномасы (CRP) онкологияның маңызды мәселелерінің бірі болып табылады, себебі жыл сайын аурудың көбеюі және өлім-жітімнің көп болуы. бүйректік жасушалық карцинома ауруы аурудың барлық патологиялары арасында пайда болу жиілігі бойынша 12-13 құрайды, орта есеппен екі жынысқа да бірдей әсер етеді.

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ИНТРАКОРПОРАЛЬНАЯ РЕЗЕКЦИЯ ПОЧКИ В УСЛОВИЯХ ХОЛОДОВОЙ ИШЕМИИ С РЕГИОНАРНОЙ ПЕРФУЗИЕЙ

Аннотация. Почечно-клеточный рак (ПКР) относится к одной из наиболее важных проблем онко-урологии, в связи с ежегодно возрастающей заболеваемостью и высоким уровнем смертности. По данным канцер-регистра заболеваемость в Республике Казахстан (РК) почечно-клеточным раком занимает 12-13 ранговые места по частоте встречаемости среди всех онкопатологий, в среднем одинаково часто поражая оба пола.

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DOUBLE-STRANDED DNA VIROME OF THE SMALL ARAL SEA

Abstract. The study of environmental biodiversity is the most important direction of research in biological disciplines. Particularly relevant is the study of degrading ecosystems, because this may indicate the direction in restoring the equilibrium in the ecosystem. The taxonomic diversity of double-stranded DNA (dsDNA) virome in the Small Aral Sea is dealt with the article. The choice of the research object is primarily due to the fact that dsDNA virome is the most numerous component of any ecosystem. Virome of the research sample was studied by multiple parallel sequencing method. As a result of the sequencing data processing, 43009 viral sequences were obtained. A comparative study of viral sequences showed that most viruses possess with dsDNA genome. It was found that among dsDNA virome both autochthonous and allochthonous viruses are present. The most numerous were autochthonous viruses that infect organisms of three evolutionary domains and belong to the *Caudovirales* order and nucleocytoplasmic viruses of the *Iridoviridae, Mimiviridae* and *Phycodnaviridae* families. Despite the small number (3%), among the allochthonous viruses, strains of 13 different families that can cause infections in humans and animals were detected, which indicates an anthropogenic impact on the Aral Sea. Studies show a large genotypic diversity of the Aral Sea viruses, and emphasize the need for more comprehensive analysis of those viruses that occur in one of the world's largest saline water ecosystems.

Key words: the Aral Sea, virome, bacteriophages.

Introduction. For the first time, aquatic viruses were recognized as pathogens of fish diseases such as pancreatic infection, necrosis and Oregon sockeye disease in the early 60s of the 20th century [1]. Since then, it has been proven that viruses affect absolutely all representatives of marine life from bacteria to protozoa, mollusks, crustaceans, fish and mammals [2]. Since the early 1990s, aquatic viruses have begun to be perceived not only as plant and animal pathogens, but also as one of the key factors in regulating interspecific interactions in ecosystems and the circulation of nutrients.

Viruses have a significant impact on the species and numerical composition of the main producers and decomposers of pyramid of numbers. Since Karl-Heinz Moebus' pioneering work on bacteriophages isolating from the waters of the North Atlantic [3, 4], research in marine viruses has developed into significant and independent direction of marine biology. Increasing interest in aquatic viruses is caused by an understanding of their key role in the balance and functioning of marine and freshwater ecosystems [5-7]. The invention of new methods and their technical improvement in detection and enumeration of marine viruses contributed to more detailed studies of their numbers and diversity [8]. It was found that viruses are the most abundant biological entities in the oceanic and marine environment [9]; reaching up to 10^8 viral particles in ml. Studies of aquatic viruses have become widespread, viruses of coral reefs [10], bottom sediments [11, 12], deep-sea biosphere [13], freshwater bodies [14] and others have been identified and studied. It has been proven that viruses are integrated inhabitants of all aquatic environments. Studies of recent decades confirm the key role of viruses in the regulation bacterial and algal mortality, in direction of their evolution, which in turn proves the indirect effect of viruses on both biogeocenosis and global biochemical cycles of oceans. The use of modern tools of molecular biology and next generation

sequencing in research of viruses and the genetic mechanisms of virus-host interaction has opened-up a huge amount of metagenomic data that showed a significant variety of aquatic viruses. Virome of aquatic ecosystems is considered the largest pool of unexplored genetic diversity on the globe, about 93% of the sequences not represented in the public databases [15]. Recently conducted metagenomic studies of 43 ocean sites identified 5,500 populations of only double-stranded DNA viruses [16]. Studies have shown that Flaviani et al found 254 unique viral phylotypes in a 250 ml sample of ocean water, even at very small scale, viral diversity can be significantly high, which confirms the difference between viruses in the world's oceans [17].

Of particular interest are studies of viral diversity in reservoirs with high salt content, one of which is the Aral Sea. In such environments, the distribution of viruses occurs along the gradient of salinity, and when the salinity level of the reservoir is above 20%, the level of the bacterial flora decreases rapidly. In this case, the number of heterotrophic nanoflagellates and infusorians decreases by approximately 25%, as a result the role of bacteriophages in the control of the number and species diversity of halophilic microbial communities increases frequently [18].

The aim of our research is to study the biodiversity of bacteriophages in the ecologically adverse region of the Aral Sea, which is the extreme salinity as soil and water. In our work, a metagenomic study of viral communities from the Bolshoi Saryshyganak Bay of the Small Aral Sea was conducted. The choice of location was due to a sharp increase the salinity of the region, which led to decrease the lake biodiversity. Critical morphological changes and progressive salinization have led to a profound change in the biological system of the sea. There has been a replacement of freshwater and brackish-water biological communities by broadly euryhaline species of marine and freshwater origin [19]. This makes it necessary to expand the research of the virome of the Small Aral Sea water basin, which is of interest from a theoretical and practical view.

Materials and methods. Virus containing water samples collecting. Water samples (10 liters) were collected into sterile containers. Samples were collected from surface of the Small Aral Sea at 1 week interval during the June 2018. The coordinates of the sampling point are 46 ° 37'22.6 "N 61 ° 28'25.6" E (figure 1).

Figure 1 – Place of the sampling point

Concentration of virus-containing samples. The seawater samples (300 L) were immediately filtered using a 300mm diameter cellulose membrane with a 3μm pore size and then filtered through a 0.22μm membrane, to remove the large organisms, such as zooplankton, phytoplankton, and bacteria. Next, the filtrate was concentrated to a volume of 500 ml using a tangential flow filtration (Vivaflow 200, Sartorius, with a total surface area 200 cm^2 of polyethersulfone membrane). To precipitate the virus particles, the concentrate was centrifuged using Beckman Coulter ultracentrifuge, Avanti J30I, at a speed of 29,000 rpm.

Isolation of nucleic acids. Nucleic acid was isolated from the obtained samples using the PureLinkViral DNA / RNA Mini extraction kit ("Invitrogen", USA) according to the manufacturer's protocol. A fluorescent dye specifically binding to a specific type of nucleic acid (double-stranded DNA, single-stranded DNA, RNA) was used to measure the concentration of nucleic acids. Quantitative measurements were performed using a Qubit dsDNA HS kit (High Sensitivity, Invitrogen, USA) according to the instructions for a Qubit 3.0 fluorimeter.

Virome libraries construction and sequencing. DNA libraries were designed using the Nextera XT DNA Sample Preparation Kit (llumina, USA) according to the manufacturer's protocol, which included the following steps: enzymatic DNA fragmentation, ligation of sequence adapters, pre-amplification of the library, selection of fractions of the desired length, clonal amplification of the selected library.

The amplified libraries were purifed with AMPure XP beads, the library's insert size was verified by Agilent 2100 Bioanalyzer and quantified using real-time PCR. Sequencing was performed on the MiSeq "Illumina" using the Kit v3 kit (300bp, paired-end read).

Metagenomic analyses. Sequencing data was analyzed using the Kaiju software, which allows for sensitive taxonomic classification of high-throughput sequencing of metagenomic or metatranscriptome samples [20].

Results and discussion. The multiple parallel sequencing of the genomic library isolated from the sample allowed to obtain a database of paired-end reads, each of which contained about 300 nucleotides. After bioinformatic processing of sequencing data, a database consisting of 658378 sequences belonging to three cellular domains and viruses was obtained. So, 75% of the sequences belonged to bacteria, 16% belonged to eukaryotes, and only 1% belonged to archaea. Viral sequences in investigated sample were identified in the amount of 7% (43009) (figure 2).

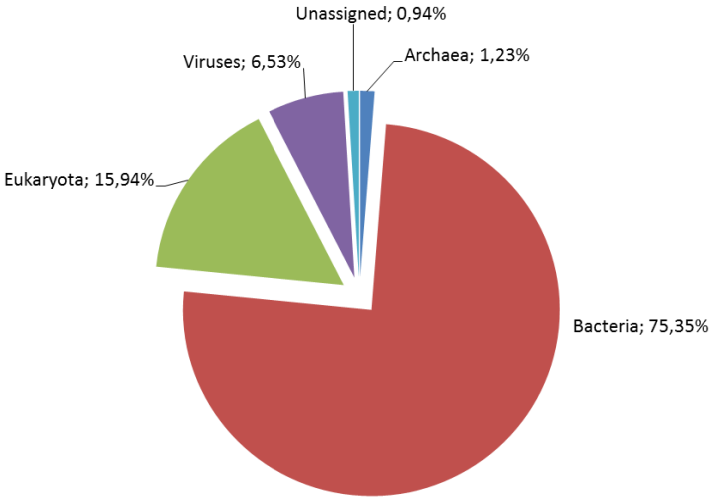


Figure 2 – The ratio of the number of sequences of the main domains

Of the 43009 viral sequences, viruses with different types of nucleic acid were identified, of which 65% were non-cultured phages and 26% were viruses with dsDNA genome (figure 3).

A comparative study of viral sequences and their belonging to host organisms showed that the dsDNA virome contains viruses of three large evolutionary domains: archaea, bacteria and eukaryotes, and consist of 3 orders, 19 families and a group of unclassified viruses (figure 4).

The dominant group of dsDNA virome were autochthonous prokaryotic viruses of the *Caudovirales* order (76%), as well as families of large nucleocytoplasmic DNA viruses, such as *Phycodnaviridae* (4.79%) and *Mimiviridae* (1.17%). Despite the apparently small number of viruses among the allochthonous viruses, 13 different families that can cause human and animal infections were detected, 2.5% of them were represented by the *Herpesvirales* order, *Iridoviridae* sequences were identified in the amount of 1%. Also there were sequences of unclassified dsDNA viruses in the amount of 11% in investigated sample (figure 4A) The remaining families capable of causing infections of humans, animals and plants were present in the water sample in an amount less than 1% (figure 4B).

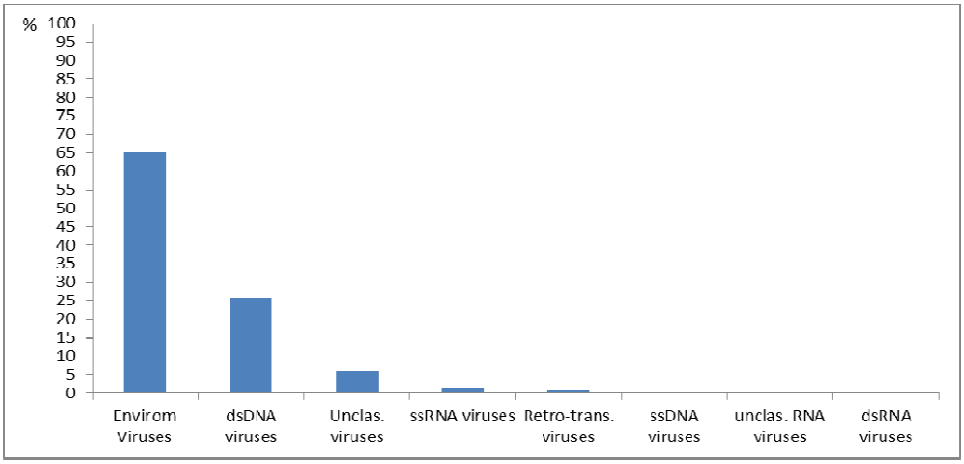
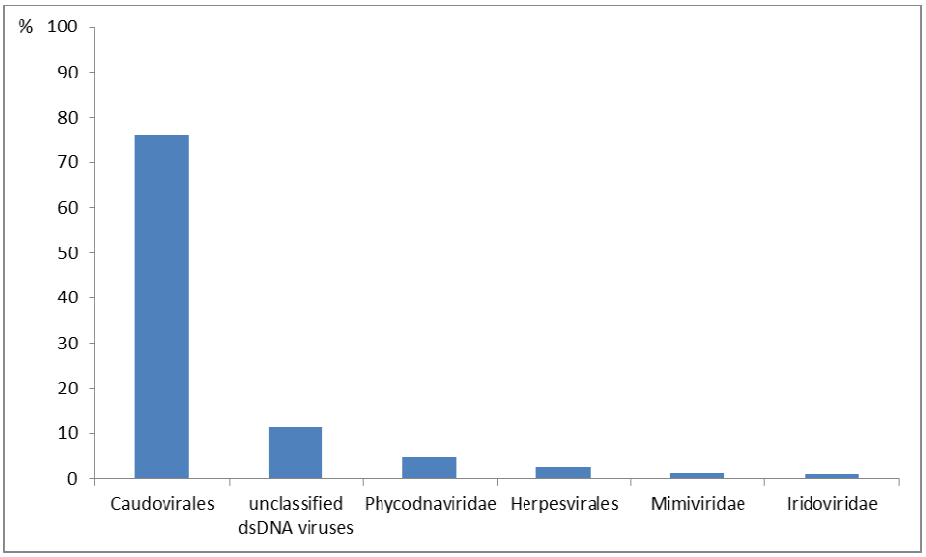


Figure 3 – The number of viral sequences with different types of nucleic acid



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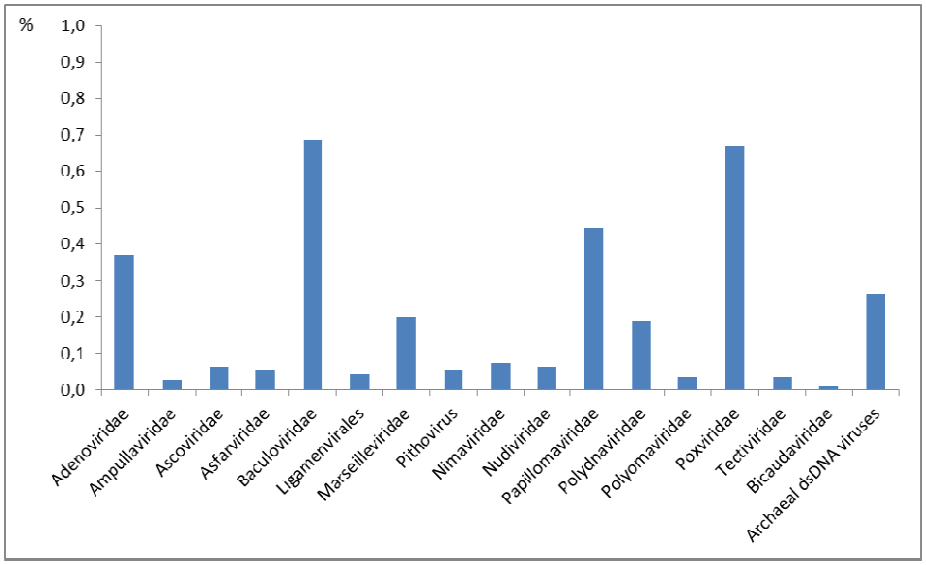


B

Figure 4 – Taxonomic composition of dsDNA virome

Among dsDNA viruses, tailed bacteriophages of the *Caudovirales* order, which contains the *Myoviridae, Siphoviridae* and *Podoviridae* families, were the dominant group (76%) in the surface waters of the Aral Sea. This result is similar to the findings of other marine viral metagenomic studies, such as Monterey Bay (65%), the Indian Ocean (95.3%), the Baltic Sea and the Antarctic Peninsula region of the Southern Ocean (~80%) in which these phages were numerically the dominant group [21-24]. *Caudovirales* were reported to infect a wide range of microbial hosts, including *Proteobacteria* and *Bacteroidetes*, which are dominant bacterial phyla in marine environments and therefore bacterial viruses (*Caudovirales* bacteriophages) are the most numerous in dsDNA virome in surface sample of the Small Aral Sea.

The percentage distribution of *Caudovirales* viruses in the studied sample was as follows: the *Podoviridae* family around 32.41%, *Siphoviridae* family around 25.38%, *Myoviridae* family 33.75% (figure 5). 9% were viruses not classified to the dsDNA family.

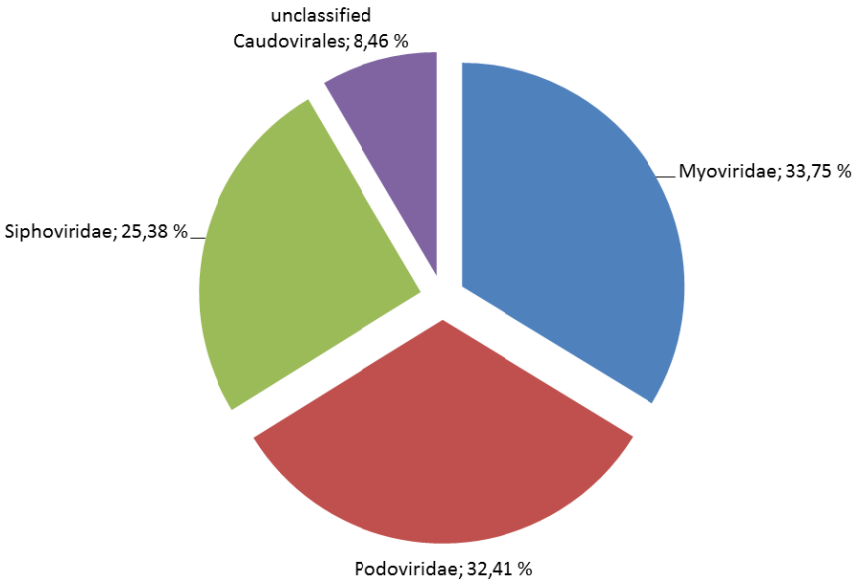


Figure 5 – Correlation of viral families of the *Caudovirales* order

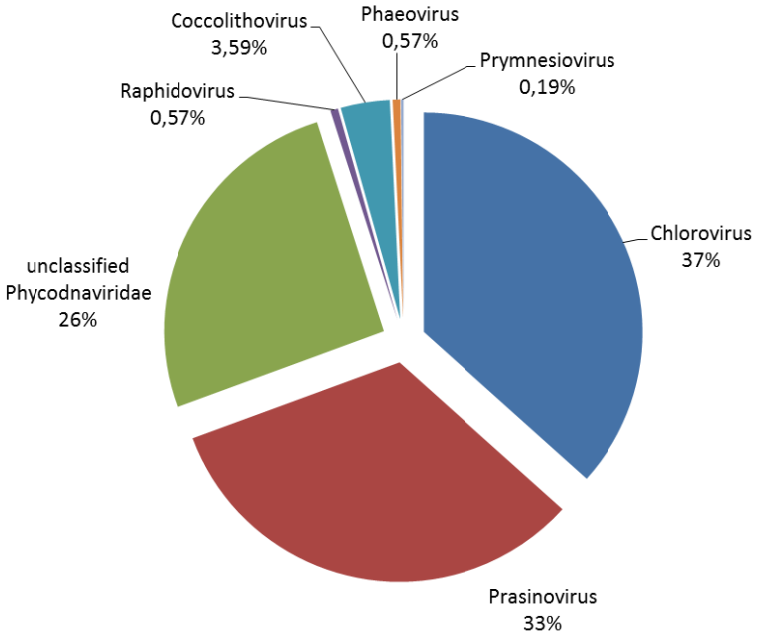
Nucleocytoplasmic DNA viruses were represented by *Phycodnaviridae* and *Mimiviridae* families.

Representatives of the *Phycodnaviridae* family that infect eukaryotic algae include the following virus genera: *Chlorovirus, Coccolithovirus, Phaeovirus, Prasinovirus, Prymnesiovirus,* and *Raphidovirus* [25]. Phylogenetic relationships between these genera are difficult to establish due to the lack of genetic data and a small number of characterized viruses in the family, which are less than three for each genus, except for *Chloroviruses*.

As a result of bioinformatic processing of sequencing data, the number of the *Phycodnaviridae* family representatives was about 5% of the total number of detected dsDNA viruses. In our sample, the sequences of all 6 genera of this family were identified, the most numerous were *Chlorovirus* genus (36.67%), Prasinovirus (32.70%), and representatives of unclassified *Phycodnaviridae* (25.71%). The remaining genera of this family were present in an amount of less than 1%: *Prymnesiovirus* (0.19%), *Phaeovirus* (0.57%), *Coccolithovirus* (3.59), *Raphidovirus* (0.57%) (figure 6).

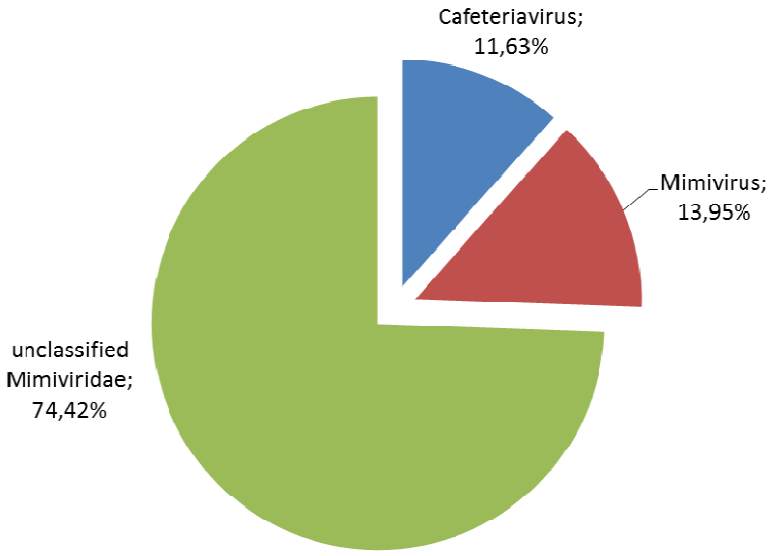
In addition, sequences of viruses infecting protozoa and belonging to the *Mimiviridae* family were identified. Recently, it was proposed to cluster mimiviruses into two genera: (1) *Mimivirus*: sub-divided into three non-taxonomical groups based on polB sequences: Group A (APMV and *Mamavirus*), Group B (*Moumouvirus*), Group C (*Megavirus chilensis*); and (2) *Cafeteriavirus*, which is a distant relation of the family *Mimiviridae* [26, 27]. In our sample, the *Mimiviridae* family was represented by *Cafeteriavirus viruses* (11.63%), *Mimivirus* (13.95%) and unclassified *Mimiviridae* (74.42%) (figure 7).

Unclassified viruses of the *Mimiviridae* family included representatives such as *Megavirus chiliensis*, *Moumouvirus*, *Niemeyer virus* and *Yellowstone lake mimivirus* in our sample.



Note. Data are given in% of the *Phycodnaviridae* family.

Figure 6 – Diversity of the *Phycodnaviridae* Family



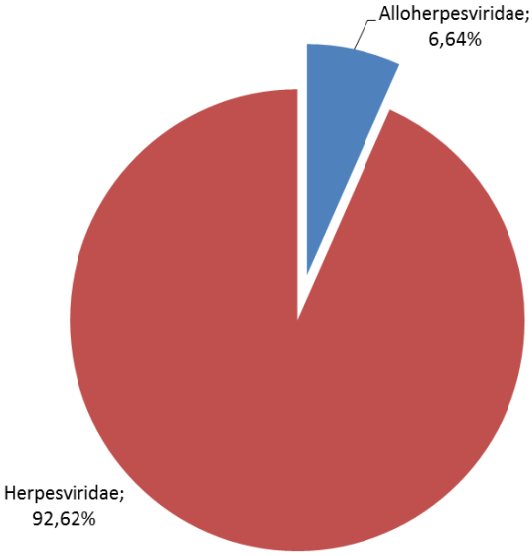
Note. Data are given in% of the *Mimiviridae* family.

Figure 7 – Diversity of the *Mimiviridae* family

In the studied reservoir, allochthonous viruses were represented by 10 families, the most numerous belonged to the *Herpesvirales* order and the *Iridoviridae* family.

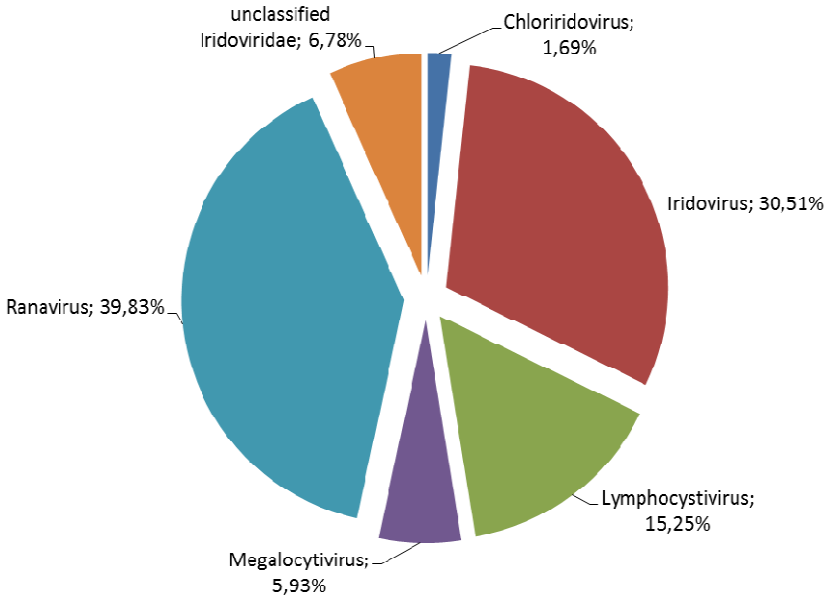
Herpesvirales is the order of DNA-containing viruses that cause a variety of diseases not only in humans and other mammals, but also in birds, reptiles, amphibians, fish. In the studied samples, this order was included two subfamilies *Herpesviridae* (93%) and *Alloherpesviridae* (7%) (figure 8).

Also, as a result of bioinformatics processing of metagenomic data, sequences of *Iridoviridae* viruses were identified, representing large icosahedral double-stranded DNA viruses that infect a wide range of both vertebrates and invertebrates (figure 9).



Note. Data are given in % of the *Herpesvirales* order.

Figure 8 – Representatives of the *Herpesvirales* order



Note. Data are given in % of the *Iridoviridae* family.

Figure 8 – Representatives of the *Iridoviridae family*

It was found that in the investigated samples identified *Iridoviridae* family consist of *Ranavirus* (40%), affecting amphibians; *Iridovirus* (30%), infecting mainly insects; *Lymphocystivirus* (15%) and *Megalocytivirus* (6%), whose host cells are fish, *Chloriridovirus* (2%), causing diseases of dipterous insects. In addition, *Scale drop disease virus* and *Anopheles minimus irodovirus* sequences related to unclassified viruses (7%) were present in the sample.

Conclusion. As a result of the research, the diversity of viral communities in the coastal waters of the Small Aral Sea was studied. It was shown that the double-stranded DNA virome of the sample contains viruses of three large evolutionary domains: archaea, bacteria, eukaryotes, and combines 3 orders, 19 families and a group of unclassified DNA viruses. It was also established that the dominant group of

dsDNA virome were autochthonous prokaryotic viruses of the *Caudovirales* order (76%), and families of large nucleocytoplasmic DNA of viruses, such as *Phycodnaviridae* (4.79%) and *Mimiviridae* (1.17%). Among the allochthonous viruses, 13 different viral families were detected, which accounted for approximately 4% of the total number of dsDNA sequences.

Thus, it was shown that autochthonous bacterial viruses represent the main virome of the sample, which indicates a rather diverse and developed population of prokaryotes in the Bolshoi Saryshyganak Bay of the Small Aral Sea. Such a population that has adapted to habitat conditions with a high salt content is the basis for the stable functioning of the local ecosystem.

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КІШІ АРАЛ ТЕҢІЗІНІҢ ЕКІ ТІЗБЕКТІ ДНҚ ВИРОМЫ

Аннотация. Қоршаған ортаның биоалуантүрлілігін зерттеу биологиялық пәндердегі зерттеулердің маңызды бағыты болып табылады. Әсіресе, тозған экожүйелерді зерттеу өзекті болып табылады, өйткені бұл экожүйенің тепе-теңдігін қалпына келтіру жөніндегі іс-қимыл бағытын көрсете алады. Мақалада Арал теңізіндегі екі тізбекті ДНҚ (дцДНҚ) виромның таксономиялық әртүрлілігі қарастырылады. Зерттеу объектісін таңдау ең алдымен дцДНҚ виромы кез келген экожүйенің ең көп құрамдас бөлігі болып табылады. Зерттелген үлгідегі виром көпше параллель секвенирлеу әдісімен зерттелді. Секвенирлеу деректерін өңдеу нәтижесінде 43009 вирусты тізбектер алынды. Вирусты тізбектерді салыстырмалы зерттеу көптеген вирустардың дцДНҚ – геномы бар екенін көрсетті. Виром дцДНҚ арасында автохтондық және аллохтондық вирустар бар екені анықталды. Ең көп болып *Caudovirales* және *Iridoviridae, Mimivridae* және *Phycodnaviridae* тұқымдастарының нуклеоцитоплазмалық вирустарына жататын үш эволюциялық домендердің ағзаларын зақымдайтын автохтонды вирустар болды. Санының аздығына қарамастан (3%) аллохтон вирустарының арасында адам мен жануарлардың инфекциясын тудыруға қабілетті 13 түрлі тұқымдастардың штаммдары диагностикаланды, бұл Арал теңізіне антропогендік әсер ететінін көрсетеді. Зерттеулер Арал теңізі вирустарының үлкен генотиптік әртүрлілігін көрсетеді,бұл әлемдегі ең көне тұзды су экожүйелерінің бірінде өмір сүретін вирустарды жан-жақты талдау қажеттілігіне әкеледі.

Түйін сөздер: Арал теңізі, виром, бактериофагтар.

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ДВУЦЕПОЧЕЧНЫЙ ДНК ВИРОМ МАЛОГО АРАЛЬСКОГО МОРЯ

Аннотация. Изучение биоразнообразия окружающей среды является важнейшим направлением исследований биологических дисциплин. Особенно актуальным является изучение деградирующих экосистем, так как это может указать направление действий по восстановлению равновесия экосистемы. В статье рассматривается таксономическое разнообразие двуцепочечного ДНК (дцДНК) вирома в Аральском море. Выбор объекта исследований обусловлен в первую очередь тем, что дцДНК виром любой экосистемы является самой многочисленной составляющей экосистемы. Виром исследуемого образца изучали методом множественного параллельного секвенирования. В результате обработки данных секвенирования было получено 43 009 вирусных последовательностей. Сравнительное изучение вирусных последовательностей показало, что большинство вирусов обладает дцДНК – геномом. Было установлено, что среди дцДНК вирома присутствуют как автохтонные, так и аллохтонные вирусы. Самыми многочисленными были автохтонные вирусы, поражающие организмы трех эволюционных доменов и принадлежащие к отряду *Caudovirales*

и нуклеоцитоплазматическим вирусам семейств *Iridoviridae, Mimiviridae* и *Phycodnaviridae*. Несмотря на кажущуюся малочисленность, (3%), среди аллохтонных вирусов диагностированы штаммы 13 различных семейств, способных вызывать инфекции человека и животных, что говорит об антропогенном влиянии на Аральское море. Исследования показывают большое генотипическое разнообразие вирусов Аральского моря, что подчеркивает необходимость всестороннего анализа тех вирусов, которые обитают в одной из древнейших в мире соленых водных экосистем.

Ключевые слова: Аральское море, виром, бактериофаги.

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FEATURES OF THE BINDING OF miR-1322 WITH mRNAs OF GENES ENCODING POLYGLUTAMINE-CONTAINING PROTEINS

Abstract. Many genes encode proteins containing polyglutamine tract, which function is not studied well. It has been established that polyglutamine expansion causes some diseases. Using the MirTarget program, we found that nucleotide sequences in the mRNA of *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A* and *POLG* genes are miR-1322 binding sites. This miRNA can bind with the sites in mRNA and suppress genes expression. There are 22, 16, 8, 17, 9 and 9 miR-1322 binding sites in mRNAs of *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A, POLG* genes, res-+pectively. These polysites encode polyglutamine from 10 to 29 amino acid residues in length. Most of the studied proteins are transcription factors and inhibition of their synthesis can cause neurodegenerative, cardiovascular and oncological diseases. MiRNA binding sites in mRNAs of orthologues genes indicate the emergence of regulation of the studied genes expression by miR-1322 many millions of years ago. Animals containing miR-1322 target genes can serve as experimental models to study the role of polyglutamine in the development of diseases.

Keywords: miR-1322, mRNA, gene, polyglutamine, disease.

Introduction. miRNAs are small non-coding RNAs that are able to regulate gene expression at post transcription level by binding with mRNAs. The role of miRNAs in different biological processes is actively being investigated. It has been shown that these molecules can act as intracellular and intercellular signaling regulators. It has been established that miRNAs bind to mRNAs in 3′-untranslated regions (3′UTRs), 5′-untranslated regions (5′UTRs) and coding domain sequences (CDSs) [1, 2]. Moreover, some miRNAs have binding sites (BS) in 5′UTRs, CDSs, and 3′UTRs [3]. The efficacy of miRNA-mediated repression increased with the number of sites [4]. It is assumed that miRNA binding to mRNA can be significant if the gene contains repeats of site sequences in coding region. Bioinformatics is actively used to fully understand, manage and analyze biological data [5]. It is possible to predict inter-actions between miRNAs and mRNAs and their properties by using different programs [6]. It has been shown that among 17,494 mRNA sequences of human genes miR-1322 has BS in 1,058 genes [7]. Most of them are located in repeat-rich coding regions of mRNAs. Depending on reading frames these BS encode polyGlu, polyAla or polySer. The objective of this study is to research the properties of miR-1322 BS in mRNAs of *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A* and *POLG* and their orthologues. These genes are involved in several diseases. Cytosine-adenine-guanine (CAG) repeat expansions in the coding regions of *ATXN1, ATXN2,* and *ATXN7* are the cause of spinocerebellar ataxias (SCAs) [8]. It has been shown that *KCNN3* may play an important role in the pathogenesis of atrial fibrillation [9]. In other study the *KCNN3* and other small conductance calcium-activated potassium channels are proposed as promising therapeutic targets for neurodegenerative disorders such as Parkinson's disease [10] It has been shown that *MEF2A* might be involved in myocardial infarction, neurodegenerative disorders and in hepatocellular carcinoma development [11, 12]. It has been shown that *POLG* can play a significant role in Parkinson's disease and tumor promotion [13, 14]. Studying of regulation mechanisms of these genes expression is a

promising area for detection and treatment of some neurodegenerative, cardiovascular and oncological diseases.

Materials and methods. The nucleotide sequences of mRNAs of *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A* and *POLG* human genes (Homo sapience – *Hsa*) and their orthologous genes (*Acinonyx jubatus – Aju, Ailuropoda melanoleuca – Ame, Balaenoptera acutorostrata scammoni – Bac, Bos mutus – Bmu, Bos taurus – Bta, Castor Canadensis – cca, Callithrix jacchus– Cja, Canis familiaris – Cfa, Capra hircus – Chi, Chlorocebus sabaeus – Csa, Cricetulus griseus – Cgr, Equus caballus – Eca, Felis catus – Fca, Gorilla gorilla – Ggo, Loxodonta africana – Laf, Lipotes vexillifer – Lve, Macaca fascicularis – Mfa, Macaca mulatta – Mml, Monodelphis domestica – Mdo, Mus musculus – Mmu, Nannospalax galili – Nga, Nomascus leucogenys – Nle, Ornithorhynchus anatinus – oan, Oryctolagus cuniculus – Ocu, Ovis aries – Oar, Pan paniscus – Ppa, Pan troglodytes – Ptr, Pteropus alecto – Pal, Pongo abelii – Pab, Pantholops hodgsonii – Pho, Rhinopithecus bieti – Rbi, Rhinopithecus roxellana– Rro, Rattus norvegicus – Rno, Saimiri boliviensis boliviensis – Sbo, Sarcophilus harrisii – sha, Sus scrofa – Ssc)* were downloaded from NCBI GenBank (http://www.ncbi.nlm.nih.gov). Nucleotide sequence of human mature miR-1322 was downloaded from the miRBase database (http://mir-base.org). The miR-1322 binding sites in CDS region of mRNAs of *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A*, *POLG* genes were predicted using the MirTarget program. This program defines the features of binding: a) the localization of miRNA BS in 5'UTR, CDS and 3'UTR of mRNAs; b) the free energy of hybridization (ΔG, kJ/mole); c) schemes of nucleotide interactions between miRNAs and mRNA. The ratio ΔG/ΔGm (%) was determined for each site (ΔGm equals the free energy of miRNA binding with its perfect complementary nucleotide sequence). miRNA BS interacting with mRNAs with ΔG/ΔGm ratio of 85% or more were considered. Described BS are polysites arranged in series. The program determines position of BS beginning from the first nucleotide of 5'UTR mRNA. The MirTarget program also takes into account the hydrogen bonds between adenine (A) and uracil (U), guanine (G) and cytosine (C), G and U; A and C [3].

Results and discussion. Using MirTarget program, miR-1322 binding polysites in CDS region of mRNAs of *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A*, *POLG* genes were detected. mRNAs and miR-1322 interaction characteristics are given in the table 1. Free energy of hybridization (ΔG) of miR-1322 with mRNAs of *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A*, *POLG* genes is within -87÷-93 kJ/mole. Probability of the interaction between mRNA and miRNA is increases with the increase in length of polysites. ΔG/ΔGm of miR-1322 binding polysites ranged from 85 to 92%.

Table 1 – Characteristics of miR-1322 polysites in mRNAs of *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A*, *POLG* genes

Gene	The position of the beginning of binding site, nt	ΔG/ΔGm, %	Oligopeptides
ATXN1	1559 - 1592 (12) 1604 - 1631 (10)	85.4 ÷ 91.7 87.5 ÷ 89.6	QQQQQQQQQQQQHQHQQ QQQQQQQQQQQQQQH
ATXN2	657 - 714 (16)	87.5 ÷ 89.6	QQQQQQQQQQQQQQQQQQQQQQQQPP
ATXN7	637 - 658 (8)	85.4 ÷ 89.6	QQQQQQQQQPP
KCNN3	401- 425 (7) 512 - 539 (10)	87.5 ÷ 91.7 87.5 ÷ 91.7	QQQQQQQQQQQPP QQQQQQQQQQQQQP
MEF2A	1836 - 1860 (9)	85.4 ÷ 89.6	GFQQQQQQQQQQP
POLG	405 - 428 (9)	85.4 ÷ 87.5	RQQQQQQQQQQQQPQ

Expansion of a polyglutamine tract within the ATXN1 causes lethal neurodegenerative disorder SCA1 [15]. Understanding the normal function of *ATXN1* is essential to decipher the pathogenesis mechanisms in SCA1. Normal alleles of *ATXN1* have a size range of 19–36 repeats, whereas pathological alleles have 39–82 repeats. Two clusters of miR-1322 polysites have been predicted in CDS of mRNA of *ATXN1*: 12 BS for miR-1322 at the position from 1559 to 1611 and 10 miR-1322 BS from 1604 to 1650 nucleotides, respectively. The region of ATXN1, which contains miR-1322 binding is flanked by conserved oligopeptides in a number of orthologs (table 2). ΔG/ΔGm value of miR-1322 interaction with the mRNA BS of *ATXN1* is in the range of 85 to 92%. All orthologs in mRNA of *ATXN1* have a decrease

Table 2 – Oligopeptides of orthologous ATXN1 proteins encoded by miR-1322 binding sites

LSQTPGHKAE**QQQQQQQQQQQQHQHQQQQQQQQQQQQQQH**LSRAPGLITP	*Hsa*
LSQTPGHKAE**QQQQQQQQQQQQHQHQQQQQQQQQQQQH**...LSRAPGLITP	*Ppa*
LSQTPGHKAE**QQQQQHQQQQQQQHQQQQQQQQQQQH**.....LLSRAPGLITP	*Pab*
LSQTPGHKAE**QQQQQQQQQQQHQHQQQQQQQQQQQH**......LSRAPGLITP	*Ptr*
LSQTPGHKAE**QQQQQQQQQQQQQQQQHH**.............LSRAPGLITP	*Mfa*
LSQTPGHKAE**QQQQQQQQQQQQQQQQH**H.............LSRAPGLITP	*Mml*
LSQTPGHKAE**QQQQQQQQQQQQQQQH**...............LSRAPGLITP	*Nle*
LSQTPGHKAE**QQQQQQQQQQQQHH**HH...............LSRAPGLITP	*Rro*
LSQTPGHKAE**QQQQQQQQQQQQH**H.................LSRAPGLITP	*Ggo*
LSQTPGHKAE**QQQQQQQQQQQQHH**.................LSRAPGLITP	*Csa*
Note: in the Table 2 and hereinafter the bold type indicates amino acids encoded by miR-1322 binding sites	

in the number of miR-1322 BS (table 2). Most orthologues mRNAs containing miR-1322 BS were revealed among primates and contained only first set of two miR-1322 polysites.

The protein encoded by the *ATXN2*, contains a polyglutamine tract, long expansion (greater than 33 repeats) of which result in SCA2. Intermediate-length expansions (27-33 glutamines) contribute to susceptibility to amyotrophic lateral sclerosis [16]. In CDS mRNA of *ATXN2* gene, 16 miR-1322 BS were identified in the region from 657 to 733 nucleotides of mRNA with an interaction value ΔG/ΔGm of 87.5-89.6%. The region of mRNA of *ATXN2*, which contains miR-1322 BS in CDS, encodes polyGlu. For the group of orthologues, polygGlu in ATXN2 protein is flanked by conservative decapeptides (table 3). Most species of *ATXN2* gene orthologs in mRNA contains decrease in the number of miR-1322 BS, except *P. troglodytes* containing 19 miR-1322 BS. *F. catus, N. leucogenys* have only five amino acids in ATXN2 protein sequences before polyglutamine tract. While studying the regulation of *ATXN2* expression by miR-1322 in mammals, difference in the number of miR-1322 polysites in mRNA of *ATXN2* gene orthologs should be taken into account.

Table 3 – Oligopeptides of orthologous ATXN2 proteins encoded by miR-1322 binding sites

YGPLTMSLKP**QQQQQQQQQQQQQQQQQQQQQQQQQPP**PAAANVRKPG	*Ptr*
YGPLTMSLKP**QQQQQQQQQQQQQQQQQQQQQQQQQPP**...PAAANVRKPG	*Hsa*
.....MSLKP**QQQQQQQQQQQQQQQQQQQQQPP**......PPAAANVRKPG	*Nle*
YGPLTMSLKP**QQQQQQQQQQQQQQQQQQQQPP**........PPAAANVRKPG	*Csa*
YGPLTMSLKP**QQQQQQQQQQQQQQQQQQPP**...........AAANVRKPG	*Mml,Mfa*
YGPLTMSLKP**QQQQQQQQQQQQQQQPQPP**...........AAANVRKPG	*Cja*
YGPLTMSLKP**QQQQQQQQPQPQP**.................AAANARKPG	*Bta*
YGPLTMSLKP**QQQQQQQQQQPQP**.................AAANARKPG	*Chi*
.....MSLKP**QQQQQQQQQPP**...................AAANARKPG	*Fca*
YGPLTMSLKP**QQQQQQQQQQPP**...................AAANARKPG	*Aju*

ATXN7 is a transcription factor that appears to be critically important for chromatin remodeling at the level of histone acetylation and deubiquitination [17]. It has been determined that the diseased allele associated with SCA7 contains 37-306 CAG, compared to 4-35 in the normal allele [18]. MRNA of *ATXN7* contains eight miR-1322 BS with ΔG/ΔGm ratio of 85 to 90%. miR-1322 BS are found in 12 mammalian species mRNAs of *ATXN7* orthologs (table 4). mRNA of human *ATXN7* contains the greatest number of miR-1322 BS. A decrease in the number of miR-1322 BS in mRNAs of orthologs was observed. So, there are seven miR-1322 BS in mRNA of *O. cuniculus*, six miR-1322 BS in mRNA of *P. abelii* and *Ch. sabaeus*, Five miR-1322 BS are predicted in mRNA of *M. musculus*, Variable in lenghth polyalanine sequence flanks polyGlu from the N-terminal in ATXN7 protein. Amino acid sequence flanking the BS from C-terminus of ATXN7 is also variable in orthologous proteins.

Table 4 – Oligopeptides of orthologous ATXN7 proteins encoded by miR-1322 binding sites

RAAAA.GGAAAAAA**RQQQQQQQQQQPP**PPQPQRQQHPPPPPRR	*Hsa*
RAAAAAGGAAAA..**RQPQQQQQQQPP**...QPQRQQ...PPPRR	*Ocu*
RAAAAAGGAAAAAA**RQQQQQQQQPP**...SQPQRQPPPPPPPPPPRR	*Cfa*
RAAAAAGGAAAAAAR**RQQQQQQQP**....SQPQRQHSPPPPPRR	*Cja*
RAAAAAGGAAAAAA**RQQQQQQQPP**.....QPQRQQQPPPPPRR	*Pab*
RAAAA.GGAAAAAA**RQQQQQQQPP**.....QPQRQQQPPPPPPR	*Csa*
RRAA..GGAAAA..**RQQQQQPQP**.....LQPQRQHPPL...RR	*Mmu*
RAAAAAGGAAAAAG**RQQQQQPP**.......QSQRQQQPPPPPRR	*Nle*
RAAAAGGAAAAAAR**RQQQQQPP**.......QPQRQQQPPPPPPR	*Mml*
RAAAAAGGAAAAAA**RQQQQQPP**.......QPQRQ..PPPP.RR	*Chi*
RAAAA.GGAAAAAA**RQQQQQPP**.......QPPQPQRQPPP.RR	*Ssc*
RRAA..GGAAAA..**RQQQQP**QP......LQLQRQ..HPPP.RR	*Rno*

KCNN3 belongs to the KCNN family of potassium channels and contains two CAG repeat regions in CDS. It has been shown that *KCNN3* SNP polymorphism significantly increases the risk of atrial fibrillation [19]. SK channels are promising therapeutic targets for Parkinson's disease [9]. It has been established that both polyGlu regions are encoded by miR-1322 BS. These two sets of polysites consist of seven and ten BS (ΔG/ΔGm is equal to 87.5 – 91.7%) located in *KCNN3* mRNA from 401 to 444 nt and from 512 to 558 nt, respectively (table 5, 6).

Table 5 – Oligopeptides of orthologous KCNN3 proteins encoded by miR-1322 binding sites located from 401 to 444 nt

KCPCPSSGDE**QQQQQQQQQQQQQQQQQQQPP**PPPAPPATPQQPPGPPL	*Laf*
KCPCPSSGDE**QQQQQQQQQQQQQQQQQQQPP**..PPAPPAAPQQPLGPSL	*Ggo*
KCPCPSSGDE**QQQQQQQQQQQQQQQQQPP**....PPAPPAAPQQPLGPSL	*Ptr*
KCPCPSSGDE**QQQQQQQQQQQQQPP**.......PPAPPAAPQQPLGPSL	*Hsa*
KCPCPSSGDE**QQQQQQQQQQQQQPP**.......PPAPPAAPQQPLGPSL	*Pab*
KCPCPSSGDE**QQQQQQQQQQQQPP**........PPAPPAAPQQPLGPSL	*Mml*
KCPCPSSGDE**QQQQQQQQQQQQPP**........PPAPPAAPQQPLGPSL	*Mfa*
KCPCPSSGDE**QQQQQQQQQQQQPP**........PPAPPAAPQQPLGPSL	*Csa*
KCPCPSSGDE**QQQQQQQQQQQQPP**........PPAPPAAPQQPLGPSL	*Nle*
KCPCPSSGDE**QQQQQQQQQQQPP**......PPPPPAPPAAPQQPPGPPQ	*Eca*
KCPCPSSGDE**QQQQQQQQQQPP**.......PPPPAPPAAPQQPPGPSL	*Sbo*
KCPCPSSGDE**QQQQQQQQQQPP**........PPPPAPPAAPQQPPGPSL	*Cja*
KCPCPSSGDE**QQQQQQQPP**..........PPPPAPPAAPQQPPGPPL	*Bta*
KCPCPSSGDE**QQQQQQPP**............PPPAPPAAPQQPPGPLL	*Nga*
KCPCPSSGDE**QQQQQQPP**............PPSAPPAVPQQPPGPLL	*Rno*
KCPCPSSGDE**QQQQQQPP**............PPPAPPAVPQQPPGPLL	*Mmu*

The change in the number of miR-1322 BS was identified in both GAU repeat-rich sequences of *KCNN3* mRNA. The increase in the first miR-1322 binding polysites was revealed in orthologues *KCNN3* mRNAs of *L. africana, G. gorilla. P. troglodytes.* The identical number of miR-1322 BS has been found in mRNAs of *P. abelii* and *H. sapiens.* The greatest number of second miR-1322 binding polysites among orthologs was found in mRNAs of *N. galili* and *P. troglodytes.* Oligopeptides flanking polyGlu sequence encoded by miRNA BS are quite conserved in many mammalian species; however, there are changes in length of polyproline sequences flanking first set of polyGlu from C-terminus of KCNN3.

Table 6 – Oligopeptides of orthologous KCNN3 proteins encoded by miR-1322 binding sites located from 512 to 558 nt

GPLLQPQPPQL**QQL**QQQQQQQQQQQQQQQQQQQQ**P**PHPLSQLAQL	*Nga*
GPSLQPQPPQL**QQQQQQQQQQQQQQQQQQQQQ**P..PHPLSQLAQL	*Ptr*
GPSLQPQPPQL**QQQQQQQQQQQQQQQQQQQQ**P....PHPLSQLAQL	*Mml, Mfa*
GPLLQPQPPQL**QQQQQQQQQQQQQQQQQQQQ**...APLHPLPQLAQL	*Rno*
GPLLQPQPPQP**QQQQ**S**QQQQQQQ**S**QQQQQQ**...APLHPLPQLAQL	*Mmu*
GPSLQPQPPQL**QQQQQQQQQQQQQQQQ**P.....PHPLSQLAQL	*Ggo*
GPSLQPQPPQL**QQQQQQQQQQQQQQQQ**P.....PHPLSQLAQL	*Hsa*
GPSLQPQPPQL**QQQQQQQQQQQQQQQ**P.....PHPLSQLAQL	*Pab*
GPPLQPQPPQL**QQQQQQQQQQQQ**P.....HPLSQLAQL	*Lve*
GPSLQPQPPQL**QQQQQQQQQQQ**P.....PHPLSQLAQL	*Csa*
GPPLQPQPLQL**QQQQQQQQQQQ**P.....PHPLSQLAQL	*Ssc*
GPPLQPQPPQL**QQQQQQQQ**P.....PHPLSQLAQL	*Bac, Eca*
GPPLQPQPPQL**QQQQQQQPQ**.....HPLSQLTQL	*Bmu*
GPSLQPQPPQL**QQQQQQQ**X**P**.....PHPLSQLAQL	*Sbo*
GPPLQPQPPQL**QQQQQQQ**P.....PHPLSQLTQL	*Oar, Bta, Chi*
GPSLQPQPPQL**QQQQQQQ**P.....PHPLSQLAQL	*Nle, Cja*
GPPLQPQPPQLP**QQQQQQ**P.....PHPLSQLQSQ	*Laf*

The protein encoded by *MEF2A* gene is a DNA-binding transcription factor of MEF2 family. MEF2A protects primary neurons from oxidative stress-induced cell damage and may be involved in various neurodegenerative-related diseases [20]. *MEF2A* is associated with myocardial infarction [21]. Overexpression of *MEF2A* is associated with hepatocellular carcinoma [22]. There are nine miR-1322 BS located in CDS of mRNA of *MEF2A* with ΔG/ΔGm of 85.4 to 89.6%. polyGlu encoded by miR-1322 BS are flanking by conserved olygopeptides PRDRMTPS from N-terminus and RQEMGRSPVDS from C-terminus of MEF2A (table 7).

Table 7 – Oligopeptides of orthologous MEF2A proteins encoded by miR-1322 binding sites

PRDRMTPS**GFSQQQQQQQQQQQQQQQQQQQQQQQ**.....ARQEMGRSPVDS	*Sha*
PRDRMTPS**GFPQQQQQQQQQQQQQQQQQQQQQPQQQQ**.....SSRQEIGRSPVDS	*Oan*
PRDRMTPS**GFPQQQQQQQQQQQQQQQQQQQQQQQ**.....ARQEMGRSPVDS	*Mdo*
PRDRMTPS**GFQQQQQQQQQQQQQQQQQQQQQP**PQPPPPPPQPPQPQPRQEMGRSPVDS	*Nga*
PRDRMTPS**GFQQQQQQQQQQQQQP**....PPQPPPQPQPQPPQPQPRQEMGRSPVDS	*Rro*
PRDRMTPS**GFQQQQPQQQQQQQQP**.....PPPPQPQPQPPQPQPRQEMGRSPVDS	*Nle*
PRDRMTPS**GFQQQQQQQQQQSQP**.....PQPQPQPQPRQEMGRSPVDS	*Eca*
PRDRMTPS**GFQQQQQQQQQQQP**.....PPPPQPQPQPPQPQPRQEMGRSPVDS	*Hsa*
PRDRMTPS**GFQQQQQQQQQQQP**.....PPPPPQPPQPQPRQEMGRSPVDS	*Cgr*
PRDRMTPS**GFQQQQQQQQQQP**.....PQPQPQAQQPQPRQEMGRSPVDS	*Sbo*
PRDRMTPS**GFQQQQQQPQQQP**.....PPQPPQPQPRQEMGRSPVDS	*Mmu*

However, there are variable amino acid sequences containing proline and glutamine residues between polyGlu and RQEMGRSPVDSL. The maximum number of miR-1322 BS among MEF*2A* orthologues were found in *S. harrisii (22), O. anatinus (22), M. domestica (21). M. musculus* has 8 BS for miR-1322 in mRNA of *MEF2A* that should be taken into account while studying the regulation of *MEF2A* expression by miR-1322 using *M. musculus.*

The protein encoded by *POLG* is the catalytic subunit of mitochondrial DNA polymerase. POLG contains a polyglutamine tract. Nine miR-1322 BS were found in mRNA of *POLG*. This region encodes polyGlu sequence in the protein. The miR-1322 BS are located from 205 to 227 nt in *POLG* mRNA, ΔG/ΔGm is equal to 85.4 – 87.5%. There is a decrease in number of miR-1322 polysites among *POLG*

Table 8 – Oligopeptides of orthologous POLG proteins encoded by miR-1322 binding sites

SSSVPASDPSDGQRR.**RQQQQQQQQQQQQPQ**QPQVLSSEGGQL	*Hsa*
SSSVPASDPSDGQRRR**RQQQQQQQQQ**P**QQPQ**.QPQVLSSEGGQL	*Ggo*
SSSVPASDPSDGQRRR**RQQQQQQQ**P**QQPQ**...QPQVLSSEGGQP	*Ptr*
SSSVPASDPSDG.RR.**RQQQQQQQQQPQ**....QPQVPSSEGGQL	*Nle*
SSSVPASDPSDGQRR.**RQQQQQQQQPQ**.....QPQVLSSEGGQL	*Ppa*
SSSVPASDPSDEQRRR**RQQQQQQQQPQ**.....QPQVPSSEGGQL	*Pab*
SSSVPASDPSDG.RRR**RQQQQQQQQPQ**.....QQPQVPSSEDGQL	*Rbi*
SSSVPASDPSDG.RRR**RQQQPQQQPQ**.........VPSSEDGQL	*Rro*

orthologues. It was identified that miR-1322 polysites exist in *POLG* mRNA in *G. gorilla, P. troglodytes, N. leucogenys, P. paniscus, P. abelii, R. bieti* and *R. roxellana.* Therefore, in further studying of regulation of *POLG* expression by miR-1322 only some animals could be used as experimental model.

Conclusion. miR-1322 BS were found in mRNAs of *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A* and *POLG*. These binding sites are identified to encode polyGlu. in the orthologous proteins of studied animal species. The number of polyGlu amino acid residues varies during the evolution of species and it was established a tendency of increasing in the length of polyGlu sequence in the proteins during evolution. *In vitro* experiments and clinical research are needed to be conducted for further validation of the interaction between miR-1322 and mRNAs of *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A* and *POLG*. The obtained results demonstrate the possibility of an involvement of miR-1322 in diseases caused by these genes. Our analysis of physicochemical properties of BS allows us to propose an adequate experimental animal for study of regulation of *ATXN1*, *ATXN2, ATXN7, KCNN3, MEF2A* and *POLG* expression by miR-1322.

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MIR-1322 ЖӘНЕ ПОЛИГЛУТАМИНІ БАР АҚУЫЗДАРДЫ КОДТАЙТЫН ГЕНДЕРДІҢ АРАСЫНДАҒЫ БАЙЛАНЫСУ САЙТТАРДЫҢ ЕРЕКШЕЛІКТЕРІ

Аннотация. Көптеген гендер полиглутаминдік трактi бар ақуыздарды кодтайды, олардың қызметі толық анықталмаған. Ұзын полиглутаминдік тізбектің синтезі кейбір аурулардың себебі болып табылатынын көрсетілген. MirTarget бағдарламасын пайдалана отырып, *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A* және *POLG* гендерінің mRNA-ындағы нуклеотидтік тізбектер miR-1322-ның байланысу сайттары екендігі анықталды. Бұл miRNA mRNA-рымен байланыса алады және геннің экспрессиясын тежейді. *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A, POLG* гендердің mRNA-сында 22, 16, 8, 17, 9 және 9 miR-1322 байланысу сайттары бар. Бұл полисайттар, ұзындығы 10-29 амин қышқылдық қалдықтарынан тұратын, полиглутаминді кодтайды. Зерттелген ақуыздардың көпшілігі транскрипциялық факторлар болып табылады, және олардың синтезінің тежеуі нейродегенеративті, жүрек-тамыр және онкологиялық ауруларға алып келуі мүмкін. Ортологиялық гендердің мРНК-да miR-1322 байланысу сайттардың болуы, зерттелген гендердің экспрессиясын реттеуінің миллиондаған жылдар бұрын пайда болғаның көрсетеді. МиР-1322-ның нысана-гендері бар жануарлар, аурулардың дамуындағы полиглутаминнің рөлін зерттеу үшін, тәжірибелік модель ретінде болуы мүмкін.

Түйін сөздер: miR-1322, mRNA, ген, полиглутамин, ауру.

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ОСОБЕННОСТИ САЙТОВ СВЯЗЫВАНИЯ miR -1322 С ГЕНАМИ, КОДИРУЮЩИМИ ПОЛИГЛУТАМИН-СОДЕРЖАЩИЕ БЕЛКИ

Аннотация. Многие гены кодируют белки, содержащие полиглутаминовый тракт, функция которого до конца не изучена. Было показано, что синтез удлиненной последовательности полиглутамина является причиной некоторых заболеваний. С помощью программы MirTarget нами было обнаружено, что нуклеотидные последовательности в мРНК генов *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A* и *POLG* являются сайтами связывания miR-1322. Эта miRNA может связываться с сайтами в mRNA и подавлять экспрессию генов. В mRNAs генов *ATXN1, ATXN2, ATXN7, KCNN3, MEF2A, POLG* содержатся соответственно 22, 16, 8, 17, 9 и 9 сайтов связывания miR-1322. Эти полисайты кодируют полиглутамин длиной от 10 до 29 аминокислотных остатков. Большинство изученных белков являются транскрипционными факторами, и ингибирование их синтеза может вызывать нейродегенеративные, сердечно-сосудистые и онкологические заболевания. Наличие сайтов связывания miR-1322 в mRNA ортологичных генов указывают на возникновение регуляции экспрессии изученных генов много миллионов лет назад. Животные, содержащие гены-мишени miR-1322, могут служить в качестве экспериментальных моделей для изучения роли полиглутамина в развитии заболеваний.

Ключевые слова: miR-1322, mRNA, ген, полиглутамин, заболевание

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BIODIVERSITYOF GREEN (*Chlorophyta*) ALGAE OF ALAKOL LAKE AND ITS SYSTEMATICS

Abstract. In Kazakhstan there are many specially protected natural territories: nurseries, national parks, reserves, sanctuaries, wildlife areas, natural monuments, botanical gardens established for the preservation of biological diversity of the state. In many of those areas the scientists-florists conducted scientific research related to the inventory of vascular plants. Despite the substantial interest for the study of flora, the research into their diversity in various nature communities are insufficient, especially the flora of water reservoirs. The algae of water reservoirs remain studied to a small extent. Nevertheless recently we have conducted the study of algae flora in the specially protected natural territories of various regions of Kazakhstan.Earlier we published systematics and species diversity of diatoms and blue greenalgaeof Lake Alakol.In the article, the authors provide research data's for the first timeinvestigate of the algal flora of Alakol lake, which flows through 15 rivers (The Urzhar, the Katynsu, the Emelkuisa, the Yrgaity, the Zhamanty, the Zhamanotkel, the Tastu etc.). The found seaweeds were divided into: 1-systematic division, 3-clas, 7-orders, 7-families, 15-species and species belonging to 13-genures. Biodiversity of specially established types of seaweed has developed and modern taxonomy has been created. In the studied lake of the algae are found cosmopolitan species in different areas Most of the species listed here are of the plankton bacterial species and some species are of benthos.

Key words: algae, plankton, benthos, systematics, lake Alakol.

Introduction. Lake Alakol is a saline drainage lake located on the Balkhash-Alakol lowland, which is located on the border of the Almaty and East Kazakhstan regions, in the eastern part of the Balkhash-Alakol Basin. More than 15 tributaries flow into the lake, of which the main are the rivers Urzhar, Katynsu, Emelkysa, Ygrajty, Zhamanty, Zhamanotkel, Tasty. The area of the lake (with islands) is 2696 square kilometers. The volume of water is 58.56 cubic km. Length-104 km. Width-52 km. Average depth-22 m. The greatest depth is 54 m. The length of the coastline is 348 km.Together with the lakes Sasykkol, Uyaly, Zhalanashkol and others, smaller, forms the Alakol lake system.In the center of Alakol there are islands: Ulken, Kishkeni Araltobe, Belkuduk, etc. The climate of the coast is sharply continental. A complex wind regime is observed above the lake. The maximum wind speed over the northern parts of the lake reaches 40-50 m/s, over the southeastern and central 50-60 m/s. The most active winds in the autumn-winter period, when the wave height can be up to 2-2,5 m.

The duration of freeze-up is about 2 months (February-March). The largest thickness of ice is 0.8 m (in February). Melting ice-April-early May. The water temperature reaches +7+ 15^0C in late May. Mineralization of water in the water varies from 1.2 to 11.6 g/l. The composition of water is chloride-sodium and chloride-sulfate-sodium. In the waters of Lake Alakol, the high content of fluorine and bromine. In 1994, the Parliament of Kazakhstan ratified the Convention on Biological Diversity, thus affirming its desire to preserve the unique richness of nature. A real step towards the implementation of these documents was the creation in 1998 of the Alakol State Reserve(http://almatyregion-tour.kz).In 2013-2015 we studied the algae flora of one of the barely studied high mountainous reservoir - the Rakhmanovskoye lake in the Katon Karagay state National natural park of the East Kazakhstan oblast for he purposes of identification of their species diversity. As a result of research the algae composition of the

lake Rakhmanovskoye was determined in which there are 249 species, varieties and forms of algae referred to four types 10 phyla, 25 orders, 45 families and 71 genera. Cyanoprokaryota-14, Chlorophyta-63, Bacillariophyta -1 7 1 , Charopyta – 1 [1-3].

The Lake Markakol is a large water reservoir of Altay located in the mountainous gap (at the altitude of 1500 m above the sea level) in the territory of Markakol state natural reserve. The Markakol hollow is surrounded by the mountain peaks of Kurchumskiy and Azutau. As a result of algae related research in Markakol lake there were discovered 129 types of algae, referred to 3 orders: Bacillariophyta - 85, Chlorophvta -41, Cyanoprokaryota - 3. The basis of algae flora of Markakol lake create the diatomic algae (Bacillariophyta) represented by 85 species from 28 genera, 18 families, 12 orders and 3 phyla [4-9]. In 2013 we conducted algae research from rivers of Zhongar Alatau of Almaty region. As a result of processing algae samples in 2013 for Baskan river in the Zhongar Alatau of the State national natural park of the Almaty region there were discovered 37 species and types of algae referred to 3 orders: diatomic - 32, the green ones -3 and blue-green ones - 2. The basis of Baskan river is created by diatomic algae (Bacillariophyta), represented by 32 speices from 11 genuses, 7 families, 6 orders and 2 phyla. The genuses of Navicula (9), Cymbella (4), Gomphonema (4), Synedra (3), Fragilaria (3) are characterized as being most abundant in genuses [10-13].The Big Chubachye Lake is the largest of the lakes of the State National Natural Park “Burabay” in the North of Kazakhstan. The average depth of the lake is 11.1 m, the maximal 33.3 m. At the lake there is a number of small islands. The lake is drain free. The water is used for the purposes for drinking potable water, for water supply for cattle and for various economic needs of Burab settlement. As a result of processing of collected samples of alg from the considered drain water reservoir in 2012-20 there were discovered 146 species and types of alg from diatomic division - 117 species, the green ones 11, the blue-green ones - 10; euglena - 2; dinophyta charophyta algae - 3 species [14-16].

Material and methods. The material of this article is elected 2015-2017. During the summer expedition time a species was collected from different points of the Alakol lake.Along the collection of algae, meteorological conditions of the water, air and water temperature were determined. The water depth is determined by the Sekki disk, water ph- universal indicator paper. The water temperature showed the sample at 22°C, and the water was Ph-7.5.In the course of the work, commonly known classical methods of hydrobotanics and algae were used (Jiyenbekov et al.). To determined of phytoplankton samples is a specific examination by M. Gollerbach and B. N. Polyansky, also by the method of N. P. Masiuk and others use Apshtain netting with diameter 45 cm is filtered by plankton grid number 76. The collected material was fixed there in 4% solution of formalin and 96% etanol [17-19]. During harvesting, the algae type, color, colony, etc. p. signs are logged. 26 algae samples from plankton, periphyton, and benthos were collected from the lake. Diatomic algae preparations are investigated by heating. Formalin-treated material is coated with glass and heated in the electric cooker. Organic cleaning of algae piglets is carried out by firing in strong acids [20-24].

In the identification of species, light microscope MBI-3 and binoculars were produced using a computer program with the binoculars Motic BA 400 microscope, and the size of the cells was obtained by using an ocular micrometer.

Results and discussion. As a result of processing algae samples collected from Lake Alakol, analysis of algae obtained from the lake was investigated and modern systematic groups were identified.They are as follows:

1-division (*Chlorophyta*), 3-klass (*Chlorophyceae, Trebouxiophyceae, Ulvophyceae*), 7–order’s (*Sphaeropleales, Trebouxiales, Oedogoniales, Chlamydomonadales, Cladophorales, Chlorellales, Chlamydomonadales*) [25, 26], 7-family (*Botryococcaceae, Cladophoraceae, Chlorellaceae, Oedogoniaceae, Selenastraceae, Scenedesmaceae, Sphaerocystidaceae*), 13 genus (*Ankistrodesmus, Botryococcus, Bulbochaete, Chlorococcum, Cladophora, Coelastrum, Scenedesmus, Geminella, Messastrum, Oedogonium, Raphidocelis, Scenedesmus, Planctococcus*)the species belong to interdisciplinary forms with the following [27, 28], 15 - species (Anagnostidis, 2001: 359-375; Berg, 1987: 97-103; Bourrelly, 1966: 551; Bruno, 1994: 369-373; Carmichael, 1990: 87-106; Edwards, 1992: 1165-1175; Gibson, 1982: 463-489; Gromov, 2000: 79) [29, 30].

Type of Alakol lake algae

№	Name of species	№	Name of species
1	*Ankistrodesmus spiralis* (W.B.Turner) Lem.	9	*Geminella ellipsoidea* (Prescott) G.M.Smith
2	*Botryococcus braunii* Kützing	10	*Messastrum gracile* (Rein.) T.S.Gar. in T.S.Gar. et al.
3	*Bulbochaete intermedia* De Bary ex Hirn	11	*Oedogonium obtruncatum* Wittrock ex Hirn
4	*Bulbochaete nana* Wittrock ex Hirn	12	*Raphidocelis subcapitata* (Korshikov) Nygaard.
5	*Chlorococcum infusionum* (Schrank) Meneghini	13	*Scenedesmus armatus* (Chodat) Chodat
6	*Cladophora glomerata* (Linnaeus) Kützing	14	*Scenedesmus quadrispina* Chodat
7	*Coelastrum microporum* Nägeli in A.Braun	15	*Volvox aureus* Ehrenberg
8	*Desmodesmus tropicus* (W.B.Crow) E.Hegewald		

Conclusion. Discussing the results, many water reservoirs, alga flora of river lakes in our country have been studied, including the Caspian Sea, Syrdarya, Ili, Baskan and Sarkand, Shar and Kokpekty rivers and algal flora and algal biological diversity of the Alakol lake were not investigated by the country's algal specialists. One of the main objectives of the UN Conference on Biodiversity Conservation, adopted in 1992 in Rio de Janeiro is to preserve biodiversity in the environment and prevent the disappearance of species. The algal diversity of the lake is the basis for this goal. The Kazakh Fisheries Research Institute and the Zoology Research Institute have not studied of Alakol Lake Algapholics by hydrobiotes and ichthyofauna.

During our special algaeological investigations, several times this scientific expedition was built. Algae samples from the northern, southern and south-western parts of the lake were removed and the second part was mixed with 4% solution of formalin and 96% solution of ethanol. A microscopic analysis was carried out to determine the types obtained in the laboratory and the study revealed the varieties of diatomaceous algae and its modern taxonomy.Moreover, we have seen in the study that the Alcohol content of some parts of Lake Alacol Lake is very rich. But in recent years, it can be seen that anthropogenic impact on the stability of lake ecosystems and biodiversity linked to the transformation of the lake into a tourist destination. In this article, the authors regulate the stability of the lake water biota, which is the wealth of algaflora. Consequently, it saves the gaseous, salinity of the water, Ph-levels, mineral composition, and biotic content.

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АЛАКӨЛ КӨЛІНІҢ ЖАСЫЛ (*Chlorophyta*) БАЛДЫРЛАРЫНЫҢ АЛУАНТҮРЛІЛІГІ ЖӘНЕ ОНЫҢ СИСТЕМАТИКАСЫ

Аннотация. Қазақстанда көптеген ерекше қорғауға алынған табиғи аймақтар кездеседі: питомниктер, ұлттық саябақтар, қорықтар, жабайы табиғи аймақтар, табиғат ескерткіштері, ботаникалық бақтар мемлекеттің биологиялық әртүрлілігін сақтау үшін құрылған. Осы салалардың көбінде флорист ғалымдар тамырлы өсімдіктерді түгендеуге қатысып, ғылыми зерттеулер жүргізді. Өсімдіктерді зерттеуге үлкен қызығушылық болсада да, әртүрлі табиғатты қорғау қауымдастықтарында олардың алуан түрлілігіне байланысты зерттеулер, әсіресе, су объектілерінің флорасын зерттеу жеткіліксіз. Су балдырларының құрамын зерттеу төменгі деңгейде қалып отыр. Дегенмен альголог ғалымдар Қазақстанның түрлі өңірлерінің ерекше қорғалатын табиғи аумақтарында балдырлар флорасын зерттеу жұмыстарын жүргізді. Осыған дейінгі мақаламызда Алакөл көлінің диатомды және көкжасыл балдырларының алуантүрлілігі мен систематикасын жариялаған болатынбыз. Бұл мақалада авторлар 15 өзендер келіп құйатын (Үржар, Қатынсу, Емелқұйса, Ырғайты, Жаманты, Жаманөткель, Тастыт.б) Алакөл көлінің альгофлорасына алғаш рет мәліметтер беріліп отыр. Табылып, анықталған балдырлар 1 бөлімге, 3 класқа,75 қатарға, 7 тұқымдасқа, 13 туысқа жататын 15 түрлері мен түр аралық формалары екендігі анықталды. Анықталған балдырлар түрлерінің биологиялық сипаттамасы жасалып, заманауи систематикасы жасалынды. Зерттелуші көлден анықталған балдырлардың көпшілігі әртүрлі су айдындарында кеңінен таралған – космополит түрлер болып саналады. Көрсетіліп отырған түрлердің көпшілігі планктондық, аздаған түрлері бентостық түрлерге жатады.

Түйін сөздер: балдырлар, планктон, бентос, систематика, Алакөл көлі.

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БИОРАЗНООБРАЗИЯ ЗЕЛЕНЫХ (*Chlorophyta*) ВОДОРОСЛЕЙ ОЗЕРА АЛАКОЛЬ И ЕЕ СИСТЕМАТИКИ

Аннотация. В Казахстане существует много особо охраняемых природных территории: питомники, национальные парки, заповедники, районы дикой природы, памятники природы, ботанические сады, созданные для сохранения биологических многообразие растений. Во многих из этих областей ученые-флористы провели научные исследования, связанные с инвентаризаций сосудистых растений. Несмотря на существенные интересы к изучению флоры, исследование их разнообразие в различных природоохранных сообществах является недостаточным, особенно флоры водоемов. Вводоросли в водоемах остаются изученными в незначительной степени.Тем не менее недавно наши специалисты альгологи провели исследование флоры водорослей в особо охраняемых природных территориях различных регионов Казахстана. Ранее нами была опубликована систематика и видовое разнообразие диатомовых и сине-зеленых водорослей озера Алаколь. В статье авторы впервые приводят данные по изучению альгофлоры 15 рек (Урджар, Катынсу, Эмелькуйса, Ыргайты, Жаманты, Жаманутkель, Тасты и. т. д.) втекающие в озеро Алакол. Список обнаруженных видов водорослей включает:15 видов, разновидностей и формы водоросли, относящиеся к 13 родам, 7 семейству, 7 порядкам, 3 классам и 1 отделу. Составлен конспект и биологическое описание обнаруженных видов водорослей и проведена современная систематика. Большинство видов водорослей, обнаруженные в исследуемых озерах относятся к космополитным формам, широко распространенным в различных типах водоемов. Подавляющее большинство обнаруженных видов относятся к планктонным, малая часть видов – бентосные.

Ключевые слова: водоросли, планктон, бентос, систематика, озера Алаколь.

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CREATING A PHENOLOGICAL DATABASE FOR COLLECTIBLE GENE POND OF MANGYSHLAK EXPERIMENTAL BOTANICAL GARDEN WITH USE OF SPECIAL COMPUTER «Feno-S» PROGRAMS

Abstract. The characteristic of the special computer program “Feno-S” created in the MEBS is intended to enter phyto-phenological information into the computer’s memory for further operative search, mathematical processing, identification of pheno-indicators of plants, printing, building histograms and phenospectra, exporting to various text and graphic formats, compiling reports and lists for given taxonomic, bioecological, decorative and phenological parameters. A description of a simplified method of collecting, systematizing and preparing phenological studies for importing into electronic databases is given. Information is presented on the composition and structure of the database formed to date, including 130 information fields of symbolic, numerical, logical and temporary type and 3919 records by year for 533 taxa from 5 systematic departments, 8 classes, 11 subclasses, 24 superorders, 49 orders, 8 suborders, 52 families and 108 genera. Lists of the quantitative distribution of plants by morphologic and systematic groups, the most representative families and genus complexes are given.

Key words: phenology, databases, computer program, statistics of series of observations, correlation, histograms.

Introduction. Phenology, as a science,was based in the second half of the 18th century by the French scientist R. Reaumur and the Swedish scientist K. Linnem; it represents the system of knowledge of seasonal natural phenomena, terms of their beginning and the reasons, defining them [1]. Present days there are many interpretations of phenology, but the most objective definition was given last century by the famous Russian phenologist A.I. Rudenko at the first All-Union Phenological Meeting: "phenology is the science which studying regularities of seasonal development of a plant and animal life, and also the phenomena of the inorganic nature in their interrelation and interaction" [1, 2].

According with V.A. Batmanov [2] classification all researches of seasonal development of the most different objects can be subdivided into theoretical and applied phenology. Depending on objectives and the industry of the national economy the author includes agricultural, forest, hunting and transport phenology in the list of the 2nd type of phenology. According to sections of knowledge it is usually selected landscape, meteo,- zoo-, hydro- and plant phenology (phytophenology).

For botanical gardens the phenological observations are one of the principal directions the introduction test which have crucial scientific and practical importance for selection economic, valuable and adapting to a local conditions assortment; diagnostics of prospects, terms of the beginning and the end of vegetation period, blossoming and fructification; creation beautiful and constantly blossoming compositions of plants for purposes of the green buildings. Many scientists-botanists coordinate such major indicators of biological stability as winter hardiness, drought resistance, morphologic isomers of elevated bodies, qualitative and quantitative indices of the generative sphere with thephenological characteristics of introduced species [3-10]. The main criterion of introduction value of taxa is recognizing degree of compliance of dynamics of seasonal development to meteorological conditions of the area of cultivation in comparison with areas of natural origin. In this time collecting of phenological materials represents

very difficult research process caused by coverage of a large number of taxa, variety of the used concepts and terms; execution of a number of field and laboratory actions: visual observation, fixing of phenophases (until 22), filling of pivot tables, creation of phenoranges, the analysis of collected materials in connection with meteorological factors of specific year of researches and places of natural growth, etc. The most complex problem for an introductory is mathematical processing of phenological materials as all seasonal phenomena should be transferred to sequence number of day in a year, beginning from 1st of January (1st of March), and after calculation of statistical parameters to return back in a date format.

Due to the intensive development of IT-technologies in practice of plant introduction for the purpose of decrease in labor input need of translation of phytophenological researches into the modern electronic programming languages containing tens of special commands and functions of work with dates and allowing not only to create the full database (BD), but also to make different graphic and text reports, to carry out statistical processing of research material and to export it to different file formats, and also to make exchange of phenological information through Internet. Therefore, since 2018, on the base of Mangyshlak Experimental Botanical Garden (MEBG) within execution of the special grant project "Development of Scientific-Methodical and Computer-Information Bases of Carrying Out Phenological Observations in Botanical Gardens of Kazakhstan for the Forecast of Prospects of Plant Introduction, Effective Saving and Use of Their Biodiversity" (2018-2020), it carries out works on creation the multi-function phenological computer program, called "Feno-S", which would be compatible to modern operating systems, graphics and text editors and containing necessary Web-applications.

Materials and methodology. Subjects of thephenological observations were species, varieties and forms of plants of the MEBG collection fund, including 1270 taxa from 250 genera and88 families [11]. Researches of seasonal rhythms of development were based on the standard concepts and terms applied in phenology [1-2, 6-8, 12-16]: 1) subject of observation; 2) seasonal phenomenon; 3) phenological date; 4) phenological phase; 5) interphase period; 6) phenological interval and 7) phenological indicator.

In the botanical centers of Kazakhstan as the main recommendation "The technique of phenological observations in botanical gardens of the USSR" is accepted [17], which was included in 1987 in structure of the book "Techniques of the introduction researches in Kazakhstan" [18] and assumed fixing of seasonal rhythms of development separately in three morphological and systematic groups of plants: grassy, deciduous and coniferous woody plants. On this system of observations for each vegetative and generative body of introduced species during vegetation was registered data with a frequency at least two weekly; and within not less than 5 years the phenological formula is fixed which reflected a plant status. All phenophases which are observed at the moment time were fixed and quantitative parameters were noted by means of a mark by digits before designations of a phenophase: I - in case of the introduction in a phenophase less than 50% of samples; 2 – more than 50%. For researches, according to "A technique of phenological observations …" [17-18], was selected not less 5 model samples of each species. Studying of plants of different age and origin was carried out separately for reflection of intraspecific phenological heterogeneity.

Complication the algorithms of mathematical processing of research material was based on G.F. Lakin's techniques [19] and B. A. Dospekhov [20]. For development of the computer program are used 4 programming languages: Microsoft Visual FoxPro 9 SP2, Visual Basic For Applications 7.0, HTML 4.0 and JavaScript API 2.1. At structure formation of DB saving of phenomaterials was provided in two formats: actually dates in 10 symbols (for example, 17.04.2019) and in numerical - as the number of calendar days before aphenophase from a reference point – (for example – 1st of January every year (98). It considerably simplified the procedure of a statistic treatment of material of researches. Correlation coefficients were calculated as the data between terms of approach of phenophases of the same plant in different years of observations, and with meteo-factors of point of an introduction.

In phenological databases taxonomical and registration information about plant was initially entered as the indication of Latin, Russian, Kazakh names, arrangements in the Garden, numbers of registration, the donor-organization, a type of initial reproductive material, etc. For simplification of input of taxonomical units in the computer program lists of genera according to R.K. Brummitt are used [21]. The phylogenetic system is used according to A.L. Takhtadzhyan's systematics [22].

Results of researches and discussion. During developing the computer program "Feno-S" two basic principles were strictly observed: 1) The phenological DB would be closely connected by identification

indicators with collection; 2) In all algorithms of program modules information processing on seasonal development would be conducted separately for three groups of plants (deciduous and coniferous woody, grassy) owing to distinction of their morphological, ecological and biological properties. For the purpose of the solution of objectives of account, registration and mathematical processing of phenodates the structure of the main menu of the program included 11 points: "File", "Editing", "Input", "Search", "Viewing", "Lists", "Phenology", "Range", "Databases", "Service" and "Reference" (figure 1).

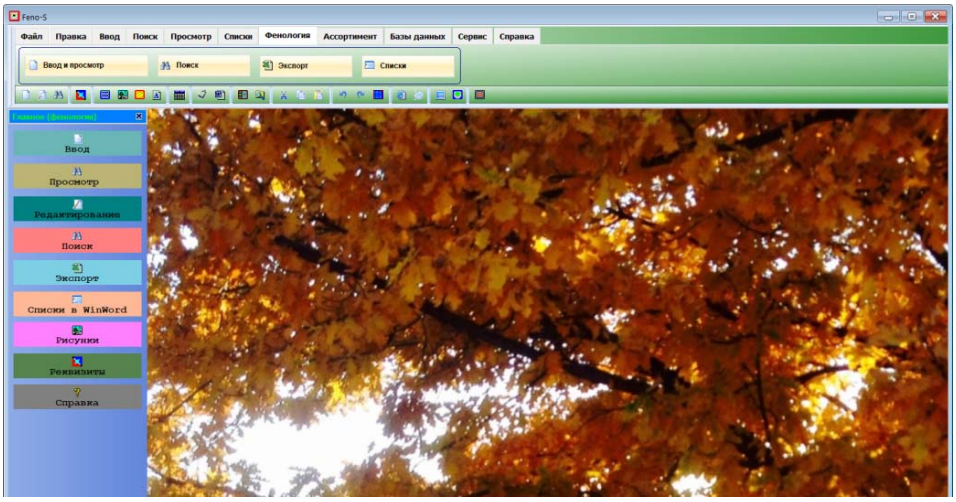


Figure 1 –The main menu of program «Feno-S»

The paragraph of Main Menu (MM) “File” includes a standard set of subparagraphs: "Open...", "My computer", "Printing", “Filer”, "Search of files", "Server", “Internet”, "Mail" and "Exit" is also intended for creation new and works with the available files, seals of information, sending it on the server and by e-mail and also an exit from the program.

The paragraph “Editing” is necessary for editing active text boxes of entry forms and viewing of information, and also for searching and replacement of words and expressions, setup of their font, color of letters and a background. From paragraph “Input” forms of filling of DB by new and editing already in-fed information are launched. "Search" allows looking for plants in DB in different options, including by any word or a fragment of a word from names.

"Viewing" is used for work with already in-fed information about plant with opportunities of its printing and export in external editors and programs in different formats - doc, docx, rtf, txt, pdf, xml, etc. Using the paragraph “Lists”, it is possible to create the most different reports about plants by taxonomical, morphological and other characteristics.

Actually four commands as "Input and Viewing", "Search", "Export" and "Lists" of point of MM “Phenology” realize a possibility of full work with information on seasonal rhythms of growth of plants as to the main objective of the “Feno-S program” (figure 1).

The paragraph of the Main Menu “Assortment” is included by three subparagraphs "On signs" "On value" and "On systematization" and is necessary for a conclusion from a DB some information on predetermined conditions. Point of MM “Data Base” is intended for implementation of the following commands: "Copying", "Restoration", "Export", "Import", “Re-indexing”, "Repair of indexes" and "Information about DB". In "Service" additional opportunities of "Feno-S" for its registration and a general setting, viewing of graphic materials and work with interactive maps on the Internet are collected.

The main entry form and viewing of phenological information is displayed in "Feno-S" at the same time with the list of Latin and Russian names of collection plants in alphabetical order (figure 2). During choosing the any taxa all information on a form is automatically updated. In the right part it is placed the list of years of the period of observations and control buttons intended for adding, copying and removal of the current year. In the lower part of a form a set of buttons for movement between years, export of information, permission of editing and saving of changes in the database is located. All phenoinformation as entered and estimated on this form is divided into 8 groups (pages): "Deciduous", "Coniferous",

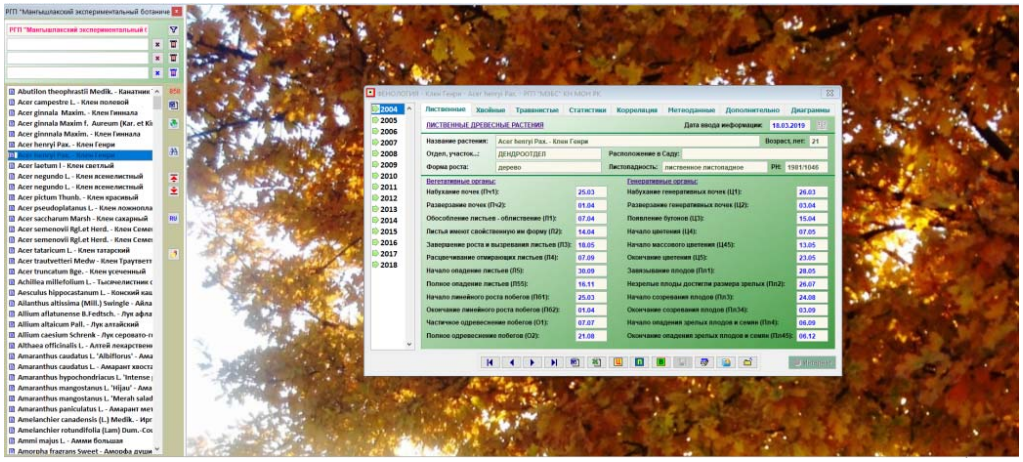
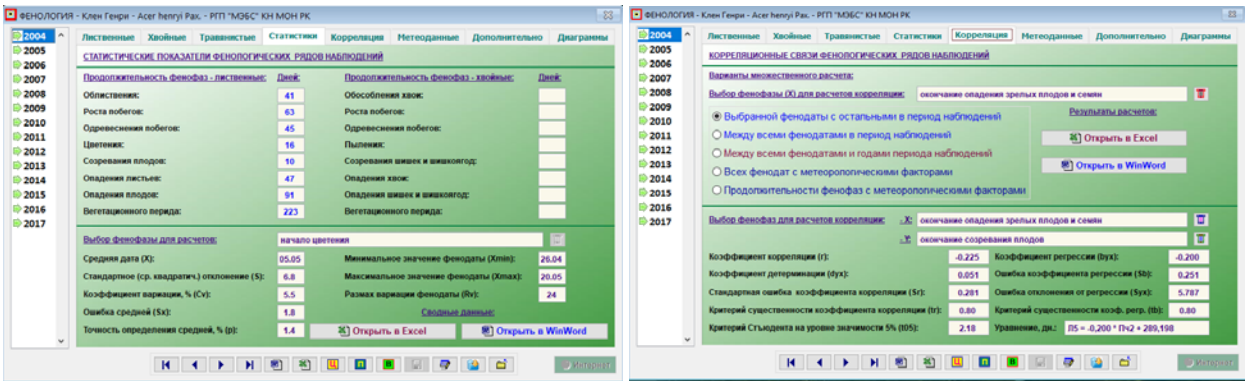


Figure 2 – Type of a form of input, viewing and processing of the phenological information (“Deciduous” page) with the list of names of plants

"Grassy", "Statistics", "Correlation", Meteodata”, "In addition" and "Diagrams". On the first three pages it is implemented the opportunity for entering and editing information for each morphological and systematic group of plants.

On the fourth page (figure 3A) introduced in an electron shell algorithms allow to count sizes of duration of phenophases (leaf opening, blossoming, growth and lignification of shoots, maturing and subsidence of fruits, etc.) and also the main statisticians for the chosen phenological phase.



A – Page «Statistics» B – Page «Correlation»

Figure 3 – Theviewofsome pages of form input and viewing

Data on multiple statistical calculations can be obtained after pressing the corresponding buttons on the lower push-button menu. At the same time at choice in Microsoft Excel or Word the table containing the following indicators (table 1) will be created: number of observations (N, years); average date in numerical (X, days) and temporary formats (X, date); standard deviation (S); coefficient of a variation (Cv); error of an average (Sx); accuracy of definition of an average (p, %), minimum (Xmin) and maximum (Xmax) date and duration of phenophases and rank of a variation (Rv, days).

The page “Correlation” (figure 3B) is intended for establishment of correlation of communication between various phenophases and meteorological factors. Here it is possible to set 5 options of correlation calculations: 1) the chosen phenodates with the others during observations; 2) between all phenodates (a correlation matrix); 3) between years of the period of observations for all phenodates; 4) all phenodates with meteorological factors (a correlation matrix) and 5) durations of phenophases with meteofactors.

The computer program in the automatic mode carries out all necessary calculations and can send at the same time the tabular report in editors of Microsoft Excel or Word with granting the following parameters (table 2): coefficients of correlation (R) and determination (D_{yx}); error of coefficient of

Table 1 – Statistics of terms of approach and duration of phenophases
for Henry's maple (*Acer henryi*Pax.) during period of observation - 2004 - 2018
(N – 15 years, T05 = 2.16)

Phenophases, duration	X, days	X, date	S	C_v	S_x	p	X_{min}	X_{max}	R_v
Phenological phases									
Vegetative organs									
Пч1–swelling of buds	94	04.04	6,9	7,4	1,8	1,9	25.03	18.04	24
Пч2–opening of buds	99	09.04	7,0	7,1	1,8	1,8	02.04	24.04	22
Л1–opening of leaves	104	14.04	7,3	7,0	1,9	1,8	06.04	30.04	24
Л2 - leaves have the form peculiar to them	109	19.04	6,7	6,1	1,7	1,6	10.04	03.05	23
Л3 - completion of growth and maturation of leaves	140	20.05	5,6	4,0	1,4	1,0	12.05	31.05	19
Л4–coloring of dying leaves	244	01.09	7,7	3,1	2,0	0,8	24.08	11.09	18
Л5 –beginning of fall of leaves	270	27.09	5,4	2,0	1,4	0,5	20.09	13.10	23
Л55 –full fall of leaves	315	11.11	8,6	2,7	2,2	0,7	25.10	19.11	25
Пб1–beginning of linear growth of shoots	111	21.04	7,9	7,2	2,1	1,9	11.04	07.05	26
Пб2–endoflinear growth of shoots	178	27.06	15,4	8,7	4,0	2,3	12.06	28.07	46
О1–partial lignification of shoots	201	20.07	16,5	8,3	4,3	2,2	07.07	20.08	44
О2–total lignification of shoots	242	30.08	12,0	5,0	3,1	1,3	20.08	23.09	34
Generative organs									
Ц1–swelling of generative buds	95	05.04	7,2	7,6	1,9	2,0	27.03	20.04	24
Ц2–opening of generative buds	102	12.04	7,2	7,2	1,9	1,9	05.04	27.04	22
Ц3 – blossoming	111	21.04	8,6	7,7	2,2	2,0	16.04	13.05	27
Ц4–beginning of flowering	126	06.05	7,4	5,9	1,9	1,5	26.04	20.05	24
Ц45 –mass flowering	130	10.05	7,5	5,8	1,9	1,5	28.04	22.05	24
Ц5 –end of flowering	139	19.05	8,4	6,1	2,2	1,6	03.05	28.05	25
Пл1–infructescence	144	24.05	4,9	3,4	1,3	0,9	16.05	31.05	15
Пл2 - unripe fruits reached the size of mature	207	26.07	2,0	0,9	0,5	0,2	22.07	30.07	8
Пл3 –beginning of maturing of fruits	237	25.08	1,9	0,8	0,5	0,2	23.08	29.08	6
Пл34 –end of maturing of fruits	250	07.09	4,4	1,7	1,1	0,4	02.09	16.09	14
Пл4 - beginning of subsidence of mature fruits and seeds	254	11.09	5,1	2,0	1,3	0,5	04.09	21.09	17
Пл45 - end of subsidence of mature fruits and seeds	335	01.12	8,6	2,6	2,2	0,7	14.11	09.12	25
Duration:									
- opening of leaves		35,7	3,3	9,2	0,8	2,2	31	41	10
- flowering		13,1	3,8	28,9	1,0	7,6	7	18	11
- growth of shoots		67,3	8,7	12,9	2,2	3,3	55	82	27
- lignification of shoots		41,4	4,8	11,7	1,2	2,9	32	46	14
- subsidenceofleaves		44,6	6,9	15,6	1,8	4,0	30	52	22
- maturing of fruits		13,2	3,8	28,6	1,0	7,6	8	22	14
- subsidence of fruits		80,9	12,1	15,0	3,1	3,8	61	96	35
- vegetation		210,8	13,7	6,5	3,5	1,7	189	227	38

Table 2 – Correlation communications of a phenophase of opening of buds (Pch2, X) of Henry's maple (*Acer henryi*Pax.) with other phenodates during observations - 2004 - 2018 (N – 15 years, T05 = 2.16)

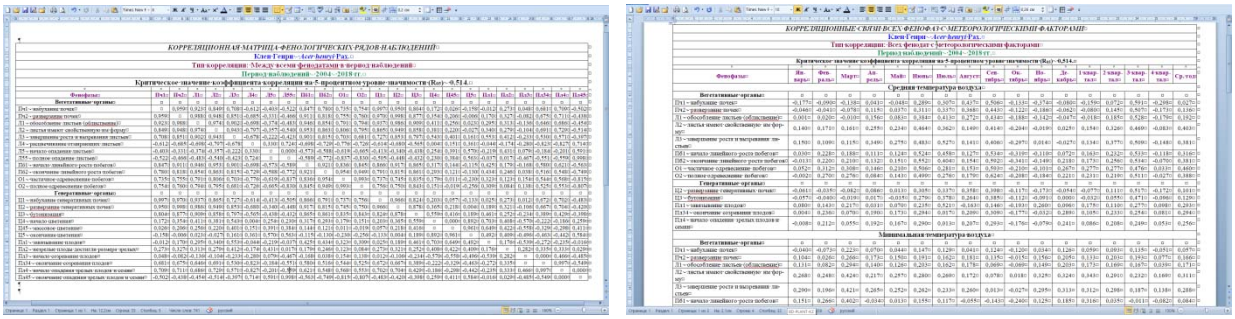
Phenophases - Y	R	D_{yx}	S_r	T_r	B_{yx}	S_b	S_{yx}	T_b
Vegetative organs								
Пч1 - swelling of buds	0,959	0,920	0,078	12,24	0,942	0,077	2,1	12,24
Л1–opening of leaves	0,988	0,975	0,044	22,66	1,018	0,045	1,2	22,66
Л2 - leaves have the form peculiar to them	0,948	0,898	0,089	10,71	0,896	0,084	2,3	10,71
Л3 - completion of growth and ripening of leaves	0,851	0,724	0,146	5,84	0,673	0,115	3,1	5,84
Л4–coloring of dying leaves	-0,685	0,469	0,202	3,39	-0,747	0,220	6,0	3,39
Л5 - beginning of subsidence of leaves	-0,331	0,109	0,262	1,26	-0,253	0,200	5,5	1,26
Л55 –endofsubsidence of leaves	-0,466	0,217	0,245	1,90	-0,571	0,301	8,2	1,90
Пб1–beginning of linear growth of shoots	0,911	0,831	0,114	7,99	1,028	0,129	3,5	7,99
Пб2–end of linear growth of leaves	0,818	0,669	0,160	5,12	1,793	0,350	9,5	5,12
О1 – partial lignification of shoots	0,755	0,571	0,182	4,16	1,775	0,427	11,6	4,16
О2 – total lignification of shoots	0,760	0,578	0,180	4,22	1,299	0,308	8,4	4,22
Generative organs								
Ц1 – swelling of generative buds	0,970	0,941	0,067	14,38	0,988	0,069	1,9	14,38
Ц2 – opening of generative buds	0,998	0,996	0,018	56,34	1,027	0,018	0,5	56,34
Ц3 – blossoming	0,877	0,769	0,133	6,58	1,067	0,162	4,4	6,58
Ц4–beginning of flowering	0,354	0,125	0,259	1,37	0,372	0,273	7,4	1,37
Ц45 –mass flowering	0,206	0,042	0,271	0,76	0,219	0,289	7,9	0,76
Ц5 –end of flowering	-0,006	0,000	0,277	0,02	-0,007	0,330	9,0	0,02
Пл1–infructescence	0,170	0,029	0,273	0,62	0,119	0,191	5,2	0,62
Пл2 - unripe fruits reached the size of mature	0,327	0,107	0,262	1,25	0,091	0,073	2,0	1,25
Пл3 – beginning of maturing of fruits	-0,082	0,007	0,276	0,30	-0,022	0,074	2,0	0,30
Пл34 – end of maturing of fruits	0,675	0,455	0,205	3,30	0,418	0,127	3,5	3,30
Пл4 - beginning of subsidence of mature fruits and seeds	0,711	0,506	0,195	3,65	0,513	0,141	3,8	3,65
Пл45 - end of subsidence of mature fruits and seeds	-0,438	0,192	0,249	1,76	-0,532	0,303	8,3	1,76

correlation (S_r); criterion of importance of coefficient of correlation (T_r); coefficient of regression (B_{yx}); errors of coefficient of regression (S_b) and a deviation from regression (S_{yx}); criteria of importance of coefficient of regression (T_b) and Student on significance value of 5% (T_{05}). Besides, in case of use of option of calculation "Chosen the phenodates with the others" along with the correlation analysis of "Feno-S" carries out removal of the equation of regression between phenodates which can be used in the future for the forecast of their approach.

The tables created as a result of export quite will approach after insignificant editing for writing of scientific articles and reports. As we see on Table 2 materials, even on the example of one species of wood plants (Henry maple - *Acer henryi*Pax.) and one type of correlation (chosen the phenodates with the others) the computer program provides to researcher considerable material for the scientific analysis.

In case of correlation calculations between all phenodates data are exported in the form of a matrix (figure 4A), and for assessment of importance of coefficients on the importance of 5% the critical value (R05) is removed them.

During calculating correlation of phenodateswith meteorological factors the program issues the tabular report on correlation communications with all 85 meteofactors in seasonal aspect, grouping them in lines on the average, minimum and maximum air temperature, relative humidity and an amount of precipitation. The report fragment in Microsoft Word on correlation of dates of approach of phenophases with average and minimum air temperature is given in figure 4B.



A – Between all phenodates B – All phenodates with meteo-factors

Figure 4 – Reports in format WinWordabout correlative bond between all phanodates and meteo-factors of *Acerhenryi*Pax. forperiod from 2004 to 2018 (N – 15 years)

The group of signs “Meteodates”, forms of input and viewing serves for input of sizes of temperature, humidity of air, an amount of precipitation, etc. On the page “Addition” there are fields of indicators of introduction value of plants, notes, names of the organization-user and performers. Points of prospects are required for establishment of correlation communications with phenological indicators of value of introduced species. On the last page of a form (figure 5) automatic creation of Gant Diagram and histograms of duration of 8 phenophases with simultaneous graphical representation in a special container is made.

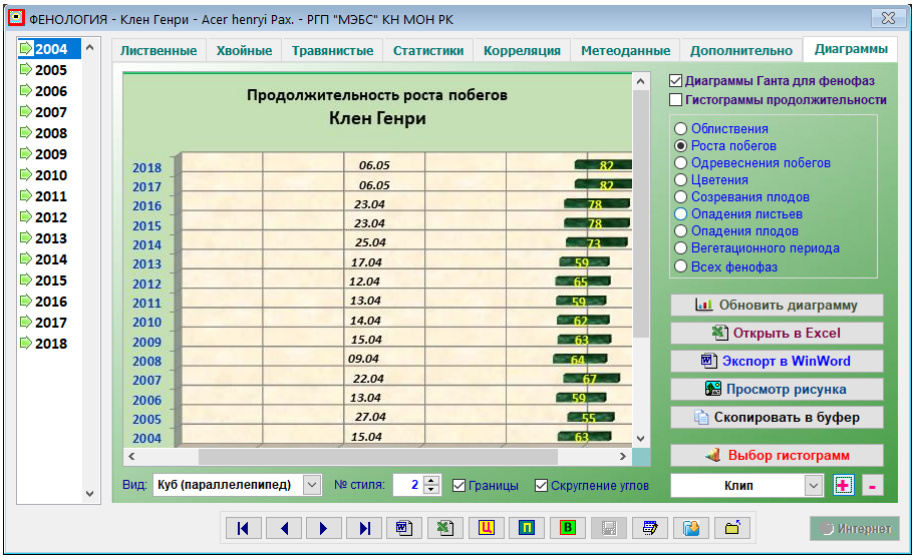


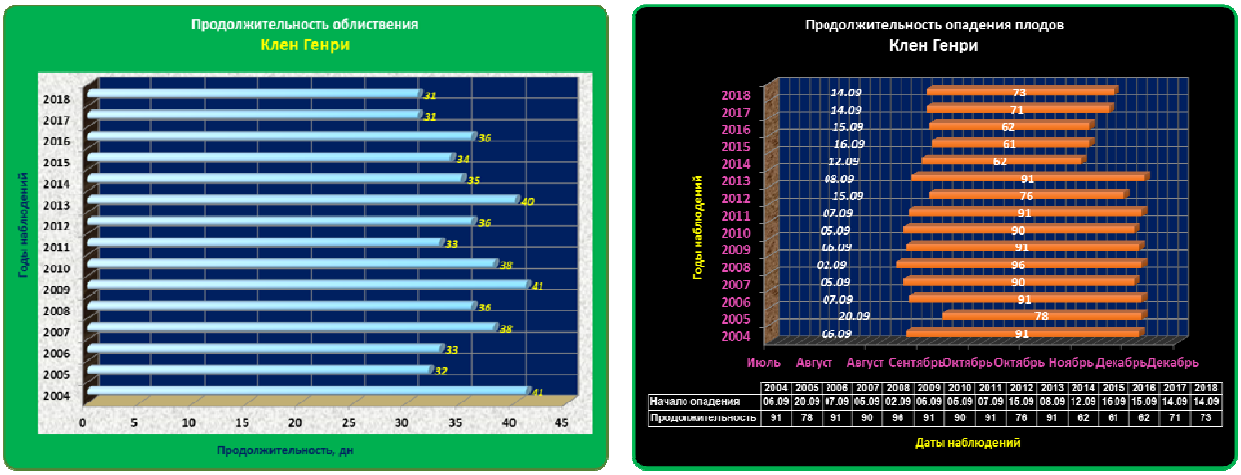
Figure 5 – Page «Diagram»

On the page “Diagram” is realized also the possibility of copying of graphic reports in a clipboard of Windows, viewing in the external editor and a conclusion in Microsoft Excel and Word.

Depending on belonging taxon groups of coniferous, deciduous or grassy plants the program can create till 17-19 graphic reports on duration of seasonal rhythms of development: leaf ability, growth and lignification of shoots, blossoming, leaf fall, maturing and subsidence of fruits, vegetative period and all phenophases (fig. 6A-6B). Here by means of button “Choice of Histograms” it is possible to start special subject to creation of chartof "FoxCharts" which allows, without leaving the program to adjust style, type of figures, color and fonts of graphic reports on phenology and also to keep them in different formats of files, including web-focused – ".html" (figure 7).

Along with drawing up a program algorithm the work on collecting, systematization and preparation for input were carried out to the spreadsheets Microsoft Excel of the long-term material of phenological researches which is saved up for the last 20 years (1999-2018) for 710 species, varieties and forms of

collection plants of a botanical garden from 58 families and 131 genera. The maximum quantity of the processed information on phenology is the share of non-local deciduous plants (dendrology department) – 417 taxa (58.7%), including 398 species, 1 variety, 12 forms and 6 hybrids. Many phenodata are systematized and prepared for a rosary (100 species and varieties, 14.1%) and department of fruit plants (83 species and varieties, 11.7%). Among families prepared for input in the editor by Excel are considerably prevailed *Aceraceae*Juss. (17 taxa), *Berberidaceae*Juss. (28), *Caprifoliaceae*Juss. (52), *Fabaceae*Lindl. (42), *Oleaceae*Hoffm. (39), *Ranunculaceae*Juss. (19), *Rosaceae*Juss. (326) and*Vitaceae*Juss. (28).



A – Histogram of duration of leaf opening B – Gant diagram of duration of subsidence of fruits

Figure 6 – Examplesofbuildingofgraphicreportsfor duration of phenophases of *Acerhenryi*Pax.

The structure of a phenological DB was developed from the used special SQL commands of the Microsoft Visual FoxPro 9 SP2 programming language and was combined with simultaneous import of the phenodata entered into the tables Excel. Initially complicated algorithm assumes selection of necessary taxonomical and registration fields from the collection database, further there is a combination

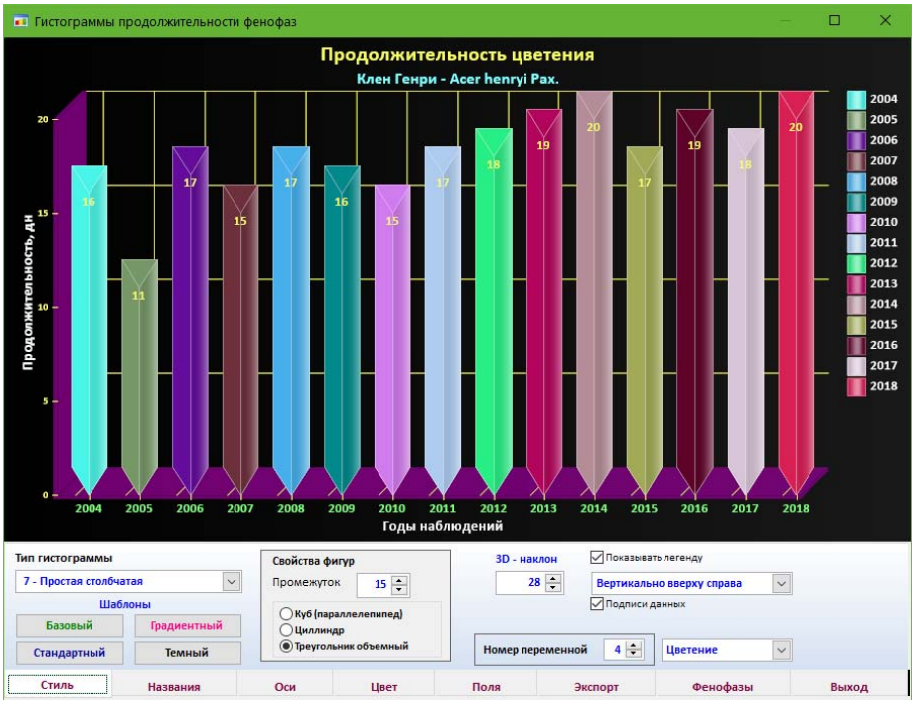


Figure7 – Form with an objects «FoxCharts» for work with histograms

of the intermediate cursor with the completed data sheet from Excel and at the last stages by a command way fields of duration of phenophases and validation of their input are added. As a result in structure of DB on phenology are created 130 fields of information of character, numerical, logical and temporary types with a total length of 2290 signs, including 6 – identification, 12 – registration, 8 – taxonomical, 33 – statistical and 60 – phenological. 11 fields are intended for characteristic of introduction value of plants on a regional complex scale. In DB for deciduous wood plants is provided storage of phenological information for 26 vegetative and generative phenophases, coniferous woody plants – 21 and grassy plants – 12. The accepted names by fields on phenophasesare corresponding to their abbreviated names according to technique of observations (the beginning of blossoming – "Ц4").

Nowadays the database contains 3919 records of phenological information by years for 533 taxa(for the period from 1 to 18 years), including 267 representatives of a dendrology department, 21 - department of Gymnospermous plants, 10 - local flora, 83 – fruit department, 100 – rosary, 31 - section of climbers and 21 – flower-decorative plants. They include 5 systematic departments, 8 classes, 11 subclasses, 24 super orders, 49 orders, 8 suborders, 52 families and 108 genera. The overwhelming quantity of taxa are species (374 - 70.2%) and varieties (138 - 25.9%). From other intraspecific ranks there are 1 subspecies, 11 forms and 9 hybrids.

The most widely taxa in the phenological database are representatives of the following families: *Aceraceae*Juss (11), *Berberidaceae* Juss. (27), *Caprifoliaceae* Juss. (32), *Fabaceae* Lindl. (15), *Oleaceae* Hoffm. (16), *Rosaceae*Juss. (291) and *Vitaceae* Juss. (24). The most numerous genera are *Acer* L. (11), *Amygdalus* L. (7), *Armeniaca* Mill. (18), *Berberis* L. (27), *Cotoneaster* Medik. (39), *Crataegus* L. (30), *Juniperus* L. (8), *Lonicera* L. (19), *Malus* Mill. (38), *Pyrus*L. (12), *Rosa*L. (111) and *Vitis* L. (18).

For carrying out correlative calculations also meteorological database is created which structure consists of 103 fields of information, 85from them are intended for data storage on meteofactors, one – actually for a year of observations and 17 - for registration and geographical indicators of the organization-user and the region of an introduction. Variables for the minimum, maximum, average temperature, relative air humidity and an amount of precipitation, as on months, and quarters and on average for a year of meteorological observations are provided in the database.

At present day works on creation of programming modules of "Feno-S" are carried out. BD is intended for identification of phenoindicators of prospects of plants, creation of phenoranges and classification of introduced species by phenorhythm type, complication of reports with multiple mathematical processing on large taxonomical units.

Conclusion. Further improvement and implementation of the phenological computer program in practice of the botanical researches considerably will simplify creation of informative databases and mathematical processing of seasonal rhythm of plant development, will allow quickly to carry out search of taxa and also will lower costs of selection of the most decorative and biologically steady range of introduced species differentiated by types of green buildings.

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ФЕНОЛОГИЯЛЫҚ ДЕРЕКТЕР БАЗАСЫН ҚҰРУ МАНҒЫШЛАҚ ЖИНАҒЫ ГРАНТТЫҚ ОРТАЛЫҒЫ БАРЫСЫНДАҒЫ ЭКСПЕРИМЕНТТІК БОТА БАСҚАРМАСЫ АРНАЙЫ КОМПЬЮТЕРДІ ПАЙДАЛАНУ «Feno-S» БАҒДАРЛАМАЛАРЫ

Аннотация. MEBS-де жасалғанарнайы «Feno-S» компьютерлік бағдарламасының сипаттамасы компьютердің жадында фитофенологиялық ақпаратты одан әрі жеделіздестіру, математикалық өңдеу, өсімдіктердің әлеуетті феноиндалитиктерін анықтау, басыпшығару, гистограммалар мен феноскраттарды анықтау, әртүрлі мәтіндік және графикалық пішімдерді, есептерді және берілген таксономикалық, биоэкологиялық, сәндік және фенологиялық параметрлері үшін тізімдерді құрастырады. Деректер базасына импорттау үшін фенологиялық зерттеулерді жинау, жүйелеу және дайындаудың жеңілдетілген әдісі сипатталған.

5 жүйелі бөлімнен, 8 сыныптан, 11 кіші сыныптан, 24 суперартраннан, 49 тапсырмадан, 8 семестрден бастап 533 таксаға арналған символикалық, сандық, логикалық және уақытша типтегі 130 ақпараттық саланы қоса алғанда, бүгінгі таңда қалыптасқан деректер базасының құрамы мен құрылымы туралы ақпарат ұсынылған. жұбайлар, 52 отбасыжәне 108 генерал. Морфо-жүйелік топтар бойынша өсімдіктердің сандық үлестірілу тізімдері, ең өкілетті отбасылар мен тұқымдық кешендер тізімі берілген.

Түйін сөздер: фенология, дерекқор, компьютерлік бағдарлама, бақылау сериясы статистикасы, корреляция, гистограмма.

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СОЗДАНИЕ ФЕНОЛОГИЧЕСКОЙ БАЗЫ ДАННЫХ ДЛЯ КОЛЛЕКЦИОННОГО ГЕНОФОНДА МАНГЫШЛАКСКОГО ЭКСПЕРИМЕНТАЛЬНОГО БОТАНИЧЕСКОГО САДА С ИСПОЛЬЗОВАНИЕМ СПЕЦИАЛЬНОЙ КОМПЬЮТЕРНОЙ ПРОГРАММЫ «Feno-S»

Аннотация. Дается характеристика созданной в МЭБС специальной компьютерной программы «Feno-S», предназначенная для ввода и хранения в памяти компьютера фитофенологической информации для дальнейшего ее оперативного поиска, математической обработки, выявление феноиндикаторов перспективности растений, вывода на печать, построения гистограмм и феноспектров, экспорта в различные текстовые и графические форматы, составления отчетов и списков по заданным таксономическим, биоэкологическим, декоративным и фенологическим параметрам. Приводится описание упрощенной методики сбора, систематизации и подготовке для импорта в электронные базы данных многолетнего материала фенологических исследований. Представлена информация по составу и структуре сформированной к настоящему времени БД, включающей 130 полей информации символьного, числового, логического и временного типа и 3919 записей по годам для 533 таксонов из 5 систематических отделов, 8 классов, 11 подклассов, 24 надпорядков, 49 порядков, 8 подпорядков, 52 семейства и 108 родов.Приведены списки количественного распределения растений по морфолого-систематическим группам, наиболее представительным семействам и родовым комплексам.

Ключевые слова:фенология, базы данных,компьютерная программа, статистики рядов наблюдений, корреляция, гистограммы.

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ISOLATION AND MOLEKULAR-GENETIC CHARACTERISTICS OF THE NOVEL AVIAN PARAMYXOVIRUS APMV-13

Abstract. The article presents the data on the isolation, identification and phylogenetic analysis of the novel avianparamyxovirus(APMV) serotype. Eighteen positive samples of APMVwere obtained during reverse transcription-polymerase chain reaction screening of 204 samples collected in five regions of Kazakhstan. The sequencing results of the L-gene fragment and BLAST analysis indicated on circulation of previously unknown avian paramyxovirus novel serotype in the populations of wild birds of Kazakhstan. Full genome sequencing of the isolate APMV-13/white-fronted goose/North Kazakhstan/5751/2013 was performed on the next generation sequencing platformHiSeq 3000 (Illumina). The sequence of genes was determined as 3'-NP-P/V/W-M-F-HN-L-5′, encoding eight proteins characteristic to the avian paramyxoviruses. Phylogenetic studies have shown that the avian paramyxovirus serotype 13is a novel natural variant, significantly different from other serotypes.

Key words: paramyxovirus, APMV-13, polymerase chain reaction, gene, sequencing, phylogenetic analysis.

Introduction. Avian paramyxoviruses (APMV) are RNA-containing viruses that form the *Avulavirus* subfamily belonging to the *Paramyxoviridae* family and can cause diseases with different clinical manifestations in most species of wild birds. According to the new classification, *Avulaviruses* on the basis of phylogenetic differences are divided into three genders –*Metaavulavirus, Orthoavulavirus, Paraavulavirus*. Until 2015, twelve serotypes of APMV (APMV-1-12) were known [1-4].

In 2015-2017 the reports were published about the discovery of seven novel serotypes of the APMV: from wild geese in Japan [5], Kazakhstan [6] and Ukraine[7], threefromducks in Japan [8], Korea [9] and from sandpiper in Brazil [10]; three more viruses were simultaneously isolated from antarctic penguins [11]. These data suggest that APMV are actively circulating in the wild avifauna and there is a high probability of the occurrence of other pathogenic variants.

To date, study of APMVs is widely conducted in various regions of the world, so a large program is carried out within the framework of the European network of excellence (EPIZONE) with the participation of many Old World countries.

Isolation and description of novel serotypes in the territory of Kazakhstan will make a significant contribution to this research.

The aim of the paper is to describe APMVs of novel serotypes circulating in Kazakhstan avian populations, to study their virological and molecular genetic features.

Materials and methods. For virological studies, samples were collected in the form of cloacal, tracheal washings from birds of water and near-water complexes. The washes were collected with a sterile cotton swab, placed in vials of medium 199 containing a complex of antibiotics (penicillin 2000 U/ml, streptomycin 2 mg/ml, gentamicin 50 μg/ml, nystatin 50 U/ml) and bovine serum albumin (0.5%/ml). For the droppings and cloacal swabs, the concentration of antibiotics was fivefold increased. Samples before virological studies were stored in liquid nitrogen (-196 °C).

Isolation and recovering passages were carried out by inoculation of each sample of the test material into the allantoic cavity of three 10-11 day embryonated chicken eggs (ECE) and then incubating at 35°C for 48-72 hours. Allantoic fluids for the presence of the virus were checked in hemagglutination(HA) test using a 0.75% suspension of chicken red blood cells. The infectious titer was calculated by the Reed-Muench method [11] and expressed in lg of $EID_{50}/_{0,2ml}$.

For removing of non-specific inhibitors of agglutination, the sera were pretreated with a receptor-destroying enzyme (RDE) from V. Cholerae filtrate (Denka Seiken Co., Ltd. Tokyo, Japan). To 1 part of undiluted serum 3 volumes of RDE were added at a working dilution of 1:50. The mixture was left at 37°C for 18 hours, then 6 parts of physiological saline was added to obtain the final dilution of the serum (1:10), and then heated at 56° C for 30 minutes.

The serotypes of APMV isolates were established in the hemagglutination inhibition (HI) test [12] with a panel of polyclonal diagnostic sera directed to: APMV-1/chicken/La Sota/46; APMV-2/Chicken/Yucaipa/56; APMV-3/Turkey/Wisconsin/68; APMV-4/duck/Hong Kong/D3/75; APMV-5/Budgerigar/Japan/Kunitachi/1975; APMV-6/duck/Hong Kong/199/77; APMV-7/dove/Tennessee/4/75; APMV-8/goose/Delaware/1053/76; APMV-9/duck/New York/22/78 provided by prof. M. Lipkind (Kimron Veterinary Institute, Beit-Dagan, Israel), additionally were updated from the National Reference Laboratory for the NDV, Friedrich-Loffler Institute, InselRiems, Germany.

RNA isolation was performed using a QIAamp Viral RNA Mini kit (Qiagen GmbH, Hilden) in accordance with the manufacturer's recommendations. RNA was extracted from 140 µl of clinical samples and eluted in a final volume of 50 µl.

The cDNA was prepared by reverse transcription reaction using the universal random hexamer primer.

Analyzes of reverse transcription PCR (RT-PCR) were performed on the basis of a one-step protocol using the appropriate RT-PCR kit (AccessQuick One-Step RT-PCR Kit, Promega) according to the manufacturer's instructions using a Pan-paramyxovirus primer to L-gene [14].

The reaction was carried out in an Eppendorf Gradient thermocycler with the following parameters: reverse transcription at 48 °C for 45 min, initial 2 min denaturation at 95 °C and amplification in 30 cycles, including denaturation (94 °C, 30 sec), primer annealing (55 °C, 30 sec) and chain extension (72 °C, 30 sec) followed by final elongation at 72 °C, 10 min.

DNA sequencing was performed using termination dideoxynucleotides on an automatic 8-capillary sequencer ABI 3500 DNA Analyzer (Applied Biosystems, USA).

For the sequencing of viral RNA on a HiSeq device (Illumina, USA), a double-stranded cDNA, which was synthesized using the RiboClone (Promega, USA) kit, was used as the template. For fragmentation of the cDNA to a size of about 250 b.p. the enzymatic method using transposase from the Nextera XT Library Preparation Kit (Illumina, USA) was used. In preparing the library of fragmented DNA, Illumina adapters were used. The quality of the prepared libraries was checked on the Bioanalyzer 2100 (Agilent Technologies, Germany). Sequencing was performed using the MiSeq Reagent v.2 kit (Illumina, USA). The resulting sequences were collected and analyzed using UGENE 1.20 software (Russia).

A TruSeq Stranded Total RNA kit with Ribo-Zero (Illumina, USA) was used to sequence viral RNA on a high-performance HiSeq 3000 device (Illumina, USA), according to the manufacturer's recommendations.

Alignment and phylogenetic analysis of sequenced genes with nucleotide sequences from Genebank was carried out using the computer program MEGA 6.0 by the method of attaching neighbors based on 1000 samples, model Tamura-Nei.

Results. Virological screening of 204 biological samples (cloacal and tracheal swabs) collected from165 bird individuals of *Anatidae, Laridae, Scolopacidae* and *Charadriidae* families of the orders *Anseriformes* and *Charadriiformes*in West, South and Central Kazakhstan in 2013was carried out to identify APMVserotypes.

APMV isolates were cloned by inoculation of 10-11 day-oldECEwith virusdiluted from 10^{-1} to 10^{-7}. The titer of virus-containing allantoic fluid in HA test at a dilution of 10^{-6} was 1:128 t - 1:512. For further molecular studies RNA was isolated from the virus suspension purified through a sucrose density gradient.

As a result of primary inoculation of 10-11 day-oldECE with samples, 20 hemagglutinating agents were isolated. PCR identification with primers to the conserved fragment of the L-geneallowed 15 agents to be assigned asAPMV.

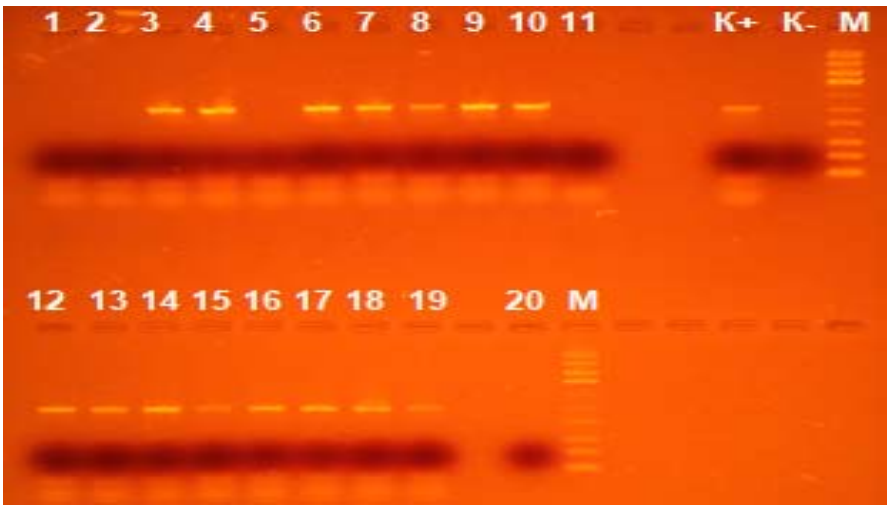
Table represents the results of HI test of APMV isolates with homologous and reference diagnostic sera.

Hemagglutinationassay results of APMV isolates from wild birds with hyperimmunerabbit and reference sera

Isolate	Immuneserumtostrain:									
	APMV-1	APMV-2	APMV-3	APMV-4	APMV-5	APMV-6	APMV-7	APMV-8	APMV-9	APMV-**13**/ WFG /North KZ/5751/2013
APMV-13/WFG*/ North Kazakhstan /5750/2013	80	0	0	0	0	0	0	0	40	**320**
APMV-13/WFG/North Kazakhstan /5751/2014	80	0	0	0	0	0	0	0	40	**320**
APMV-13/WFG/North Kazakhstan/5753/2014	80	0	0	0	0	0	0	0	40	**320**
APMV-13/pintail/North Kazakhstan/5759/2014	80	0	0	0	0	0	0	0	40	**320**
*White fronted goose.										

As can be seen from Table, the hemagglutinating activity of the Kazakhstan APMV isolates, including APMV-13/white-fronted goose/North Kazakhstan/5751/2013, were inhibited by homologous immune serum (1: 320),and they did not react or reacted in low titers with reference sera against to viruses of serotypes 1-9.

As a result of PCR specific 700 b.p.products of paramyxovirus L-gene were amplified in 15 samples.



Note: "M" is the DNA marker; "K +" - positive control; K- »- negative control; No. 1-20 of the sample number.

Figure 1 – Results of PCR with RNA from materials from wild birds of Western Kazakhstan

Sequencing of L-gene amplification products and subsequent BLAST analysis in GenBank indicated the belonging of four of them to APMV-1, six to APMV-8 and one to APMV-6. Sequence analysis of the four remaining unidentified APMV isolates of 2013 showed their significant genetic divergence by conservative fragment of the L gene with the known serotypes of the APMV (figure 2), suggesting that novelhither to unidentified APMV circulate in waterfowls of Kazakhstan.

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

	Max score	Total score	Query cover	E value	Ident	Accession
Avian paramyxovirus 12 isolate Wigeon/Italy/3920_1/2005, complete genome	289	289	91%	3e-74	73%	KC333050.1
Newcastle disease virus isolate chicken/BYP/Pakistan/2010, complete genome	143	143	91%	2e-30	67%	JN682210.1
Newcastle disease virus isolate NDV2K35/CH/TN/2003, complete genome	140	140	91%	3e-29	67%	KF740478.1
Newcastle disease virus strain cormorant/US(WI)/18719-03(USGS)/2003, partial genome	140	140	91%	3e-29	67%	GQ288385.2
Pigeon paramyxovirus 1 strain PPMV-1/Belgium/03-05843/2003, partial genome	138	138	66%	1e-28	70%	JX901118.1
Newcastle disease virus isolate chicken/CP/Pakistan/2010, complete genome	134	134	91%	1e-27	67%	JN682211.1
Newcastle disease virus isolate 2009_Mali_ML008, complete genome	132	132	83%	4e-27	67%	JF966387.1
Newcastle disease virus strain chicken/Sukorejo/019/10, complete genome	131	131	91%	2e-26	66%	HQ697255.1
Newcastle disease virus strain cormorant/US(CA)/92-23071/1997, partial genome	131	131	91%	2e-26	67%	GQ288388.2
Newcastle disease virus strain cormorant/Canada/95DC2345/1995, partial genome	131	131	91%	2e-26	67%	GQ288384.2

Figure 2 – BLAST-analysis of nucleotide sequences of unidentified Kazakhstan isolate APMV/White-fronted goose/North Kazakhstan/5751/2013

Analyzing of the L-gene of unidentified isolate APMV/White-fronted goose/North Kazakhstan/5751/2013) demonstratetheir most similarity (73%) to the referencestrain of APMV serotype 12 [13], with the remaining viruses from Genbank, the divergence index was more than 33%, which presumably attributed this strain to thenovel serotype.

In bioinformatic analysis, the obtained sequences were preliminarily assembled using the CLC Assembly Cell software (Qiagen, USA), (figure 3).

Figure 3demonstrates that the nucleotide sequences of all six APMV-13 genes were obtained in the following order: 3'-NP-P/V/W-M-F-HN-L-5 ', which encode eight proteins: NP (493 amino acids (aa), P (397 aa), V (241 aa), W (150 aa), M (366 aa), F (545 aa), HN (549 aa), and L (2199 aa).

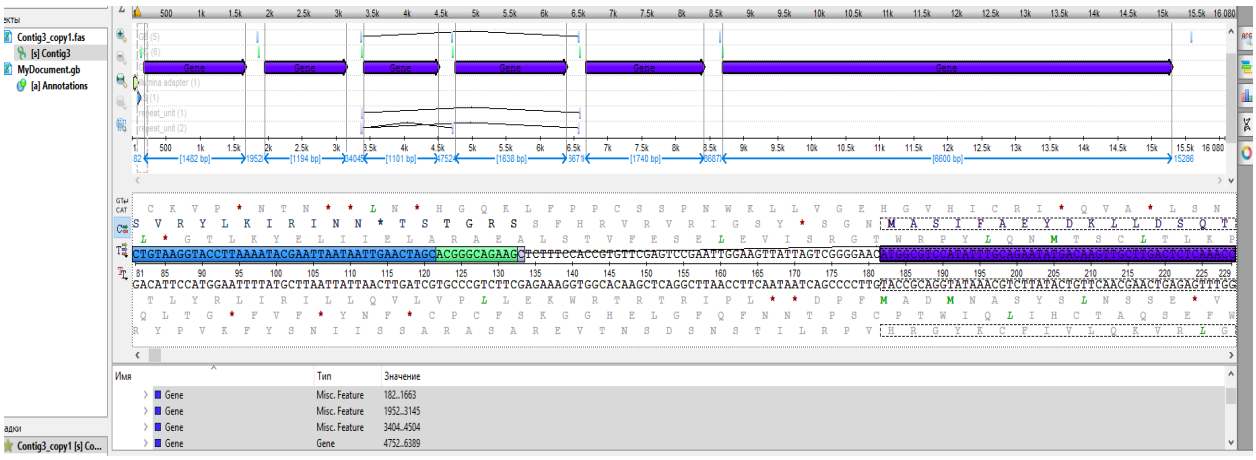


Figure 3 – View of full sequenced genome of APMV-13/ white-fronted goose/North Kazakhstan /5751/2013 in UGENE program

Next Generation sequencingof full genome of isolates and subsequent BLAST analysis identified as novel APMV serotype13.

The results of phylogenetic analysis of novel Kazakhstan APMV with representatives of serotypes 1-12 from GenBankare shown in figure 4.

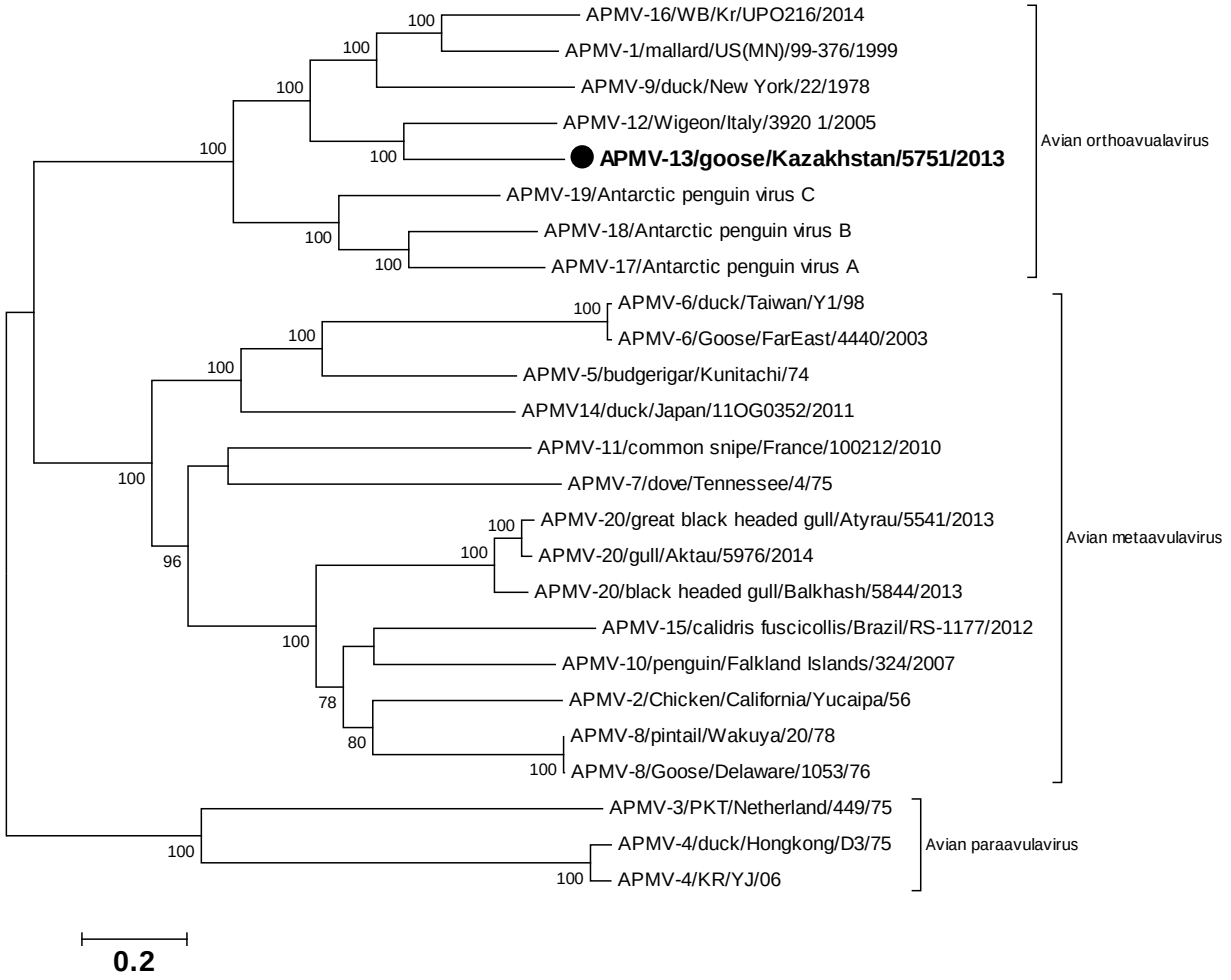


Figure 4 – Phylogenetic relationship of the novelavian paramyxovirus
APMV-13/White-fronted Goose/Northern Kazakhstan/5751/2013 with other avian paramyxoviruses serotypes

As it can be seen in Figure 4, the Kazakhstan isolate APMV-13, together with the APMVserotypes 1,9,12 and 16, formed a separate monophyletic group, within which the most phylogenetically similar was APMV-12, isolated in 2012 in Italy.

Thus, as a result of molecular genetic studies, data on the circulation of novel avian paramyxovirus serotype 13 were confirmed in Kazakhstan (according to the new taxonomic classification from 2017).

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ҚҰС ПАРАМИКСОВИРУСТАРЫНЫҢ ҒЫЛЫМҒА ЖАҢА ПМВ-13 ТҮРІН БӨЛУ ЖӘНЕ МОЛЕКУЛАЛЫ-ГЕНЕТИКАЛЫҚ СИПАТТАУ

Аннотация. Мақалада құстардың жаңа серотүрін бөлу, ажыратып балау мен филогенетикалық талдау нәтижелері сипатталады. Қазақстанның бес облысынан жиналған 204 сынаманы кері транскрипция - полимеразды тізбекті реакция скринингтеу нәтижесінде 15 нұсқасы парамиксовирустарға оң нәтиже берді. L-генінің бөлігін секвендеу әдісімен және келесілік BLAST-талдау нәтижесінде Қазақстандағы түз құстары популяциясында ПМВ белгісіз түрінің айналымда жүргенін айғақтайтын мәліметтер алынды. Соңғы үлгідегі HiSeq 3000 (Illumina) секвенаторында қазақстандық APMV-13/white-fronted goose/North

Kazakhstan/5751/2013 бөліндісі геномын толық секвендеу жүргізілді. Құс ПМВ тән, сегіз ақуыз кодтайтын 3'-NP-P/V/W-M-F-HN-L-5' гендерінің тізбегі анықталды. Филогенетикалық зерттеу нәтижесі қазақстандық ПМВ жаңа 13-серотүрі табиғи жаңа нұсқа болып саналады және өзге серотүрлерден едәуір айырмашылығы бар.

Түйін сөздер: парамиксовирус, APMV-13, полимераздытізбекті реакция, ген, секвендеу, филогенетикалық талдау.

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ИЗОЛЯЦИЯ И МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКАЯ ХАРАКТЕРИСТИКА НОВОГО ДЛЯ НАУКИ ПАРАМИКСОВИРУСА ПТИЦ APMV-13

Аннотация. В статье приведены результаты изоляции, идентификации и филогенетического анализа парамиксовируса (ПМВ) птичнового серотипа. При скрининге 204 образцов, собранных в пяти областях Казахстана в обратной транскрипции-полимеразной цепной реакции, обнаружены 18 положительных на ПМВ проб. Методом секвенирования фрагмента L-гена и последующего BLAST-анализа показана циркуляция в популяциях диких птиц Казахстана ПМВ птичнового ранее неизвестного серотипа. На секвенаторе нового поколения HiSeq 3000 (Illumina) проведено полногеномное секвенирование казахстанского изолятаAPMV-13/white-frontedgoose/NorthKazakhstan/5751/2013. Определена последовательность генов 3'-NP-P/V/W-M-F-HN-L-5', кодирующих восемь белков, характерных для ПМВ птиц. Филогенетические исследования показали, что казахстанский изолят ПМВ серотипа-13 является новым природным вариантом, значительно отличающимся от других серотипов.

Ключевые слова: парамиксовирус, APMV-13, полимеразная цепная реакция, ген, секвенирование, филогенетический анализ.

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SUCCULENT PLANTS IN THE COLLECTION OF THE INSTITUTE OF BOTANY AND PHYTOINTRODUCTION IN ALMATY

Abstract. The article “Succulent plants in the collection of the Institute of Botany and Phytointroduction of Almaty” presents the results of the introduction work with the succulents of our collection. Promising and commercially profitable plants from succulents were chosen, since they are not whimsical in care, and as a result of evolution, they easily adapt to a more or less arid climate and can be widely used. In this publication, we talked about the birth of succulents, interesting for phytodesign.

Keywords: succulent, introduction, closed ground, collection, phytodesign.

An important step in the introduction of greenery plants is the study of individual taxonomic groups (genera, species), which allows for introduction on the basis of a careful and comprehensive account of their biological, morphological and environmental features. As a result, it is possible to predict the possibility of introducing certain species into the culture and to enrich the collection gene pool of greenhouse.

Purpose: The introduction of succulent plants in the collection of the Institute of Botany and Phytointroduction.

The collection of succulents at the Institute of Botany and Phytointroduction has a long history beginning in 1932. Most of the plants were grown from seeds and live plants coming from the gardens of Germany, the Czech Republic, Brazil and other countries. Some of the plants were obtained by exchange from flower growers. The most active period of increasing the collection was from 1972 to 1982. Thanks to the peculiar structure of the plant, they are not comparable with any other group. They can exist in extreme conditions (for example, with a lack of organic matter in the soil, water in the soil and air, as well as temperature differences). As a result, the evolution of the morphological structure of succulent plants can adapt to a more or less arid climate. The distribution area of succulent plants: deserts and semi-deserts of South Africa, Mexico, Central America, mountainous areas of South America [1-3].

Succulent plants are divided, depending on the method of accumulation of water, leaf, stem and root. In the collection of the greenhouse of the Institute of Botany and Phytointroduction, there are all three varieties of succulents. Among them there are also “hard-leaved” and “herbaceous” ones.

According to the results of the introduction observations, a group of 125 species of succulents was collected and taxonomic, consisting of 47 genera belonging to 17 genus: *Agavaceae* – 17 *species, Aloaceae* – 16*, Amaryllidaceae* – 9*, Apocynaceae* – 1*, Aroliaceae* – 1*, Asclepiadaceae* – 6*, Asparagaceae* – 4*, Asteraceae* – 5*, Commelinaceae* – 2*, Crassulaceae* – 43*, Cucurbitaceae* – 1*, Draceanaceae* – 3*, Euphorbiaceae* – 12*, Hyacinthaceae* – 1*, Portulacaceae* – 2*, Urticaceae* – 1*, Vitaceae* – 1.

The most numerous genus: *Crassulaceae, including 43 species and Agavaceae – 19* (table).

For many years, the introduction work has allowed us to choose certain conditions for plants to grow. Most of the succulent species in our greenhouse grow well, blossom and bear fruit. The microclimate in the greenery: in winter the temperature reaches 10-12 °C, in spring-summer period it is 40-50 °C, the illumination on average is 80,000 lx. At low air humidity we produce irrigation with water. Plants grow in the soil and containers on the shelves in the "dry" exposure of "Arid plants". For them, an appropriate soil

Family, genus and number of species of succulents of the Institute of Botany and Phytointroduction

Family	Genus	Quantity
Agavaceae	*Agave* L.	10
	Yucca L.	1
	*Nolina*Michauz.	2
	*Dasylirion*Zucc.	1
	*Titanopsis*Schwantes.	1
	*Faucaria*Schwantes.	1
	*Ruschia*Schwantes.	1
	*Trichodiadema*Schwantes.	1
	*Corpuscularia*Schwantes.	1
Aloaceae	*Aloe* L.	3
	*Gasteria*Duval.	5
	*Bowiea*Harv. Ex Hook.f.	1
	*Hawortia*Duval.	7
Amaryllidaceae	*Haemanthus*L.	1
Apocynaceae	*Pachypodium*Lindl.	1
Aroliaceae	*Cussonia*Thunb.	1
Asclepiadaceae	*Ceropegia*L.	2
	*Stapelia*L.	1
	*Sarcostemma*R. Br.	1
	Hoya R. Br.	1
	*Huernia*R. Br.	1
Asparagaceae	*Albuca*L.	1
	*Drimiopsis*L.	1
	*Nolina*Michauz.	2
Asteraceae	*Senecio*(Tourn.) L.	5
Commelinaceae	*Tradescantia*L.	1
	*Cyanotis*D.Don.	1
Crassulaceae	*Crassula*L.	4
	Cotyledon Thunb.	1
	*Pachyphytum*Klotzschet Otto	2
	Sedum L.	6
	*Echeveria*DC.	10
	*Monanthes*Haw.	1
	*Graptopetalum*Rose.	1
	*Kalanchoe*Adans.	14
	*Sinocrassula*A.Berger.	1
	*Adromischus*Lem.	2
	*Aeonium*Webb et Berth.	1
Cucurbitaceae	*Neoalsomitra*Hutch.	1
Draceanaceae	*Sansevieria*Thunb.	3
Euphorbiaceae	*Euphorbia* L.	10
	*Monadenium*Pax.	1
	*Syandenium*Boiss.	1
Hyacinthaceae	*Ornithogalum*L.	1
Portulacaceae	*Portulacaria*L.	1
	*Ahacampseros*L.	1
Urticaceae	*Pilea*Lindl.	1
Vitaceae	*Cissus*DS.	1

composition was selected, consisting of sod-earth, sand, and humus at a ratio of 1:2:0.5. The above environmental conditions of closed ground allow plants to always remain highly decorative and multiply.

We carry out reproduction work mainly vegetatively, since not all plants under the conditions of the greenhouse undergo a full cycle. The collection is constantly updated with new species. Due to its unpretentiousness to the content, as well as high ornamental, these plants are popular among the population and are actively used in design.

When creating a collection of greenhouse, the main task was to present the morphological diversity of the species of succulents. As a result of many years of work, we selected promising plants for scientific study, as well as for commercialization. In the course of the selection, we identified the most interesting succulents according to the above principles from our collection [4].

An extensive family of the of Agave (Agavaceae), which includes 9 genus and is represented by 19 species. Of this family Agave L. is the most numerous genus. These are typical representative succulents that have rosettes of leaves with spines covered at almost all edges, the stem is very short. The leaves accumulate and store water in a viscous, colloidal state, which prevents its rapid evaporation in hot weather. The plant blooms once in a lifetime, then dies, but leaves a lot of "babies." Types of agaves are widely used in traditional medicine and cosmetology.

Picture 1 – *Agave victoriae-reginae* T.Moore

Picture 2 – *Nolinarecurvata* (Lem.) Hemsl

One of the perspective plants, also widely used in design, belongs to the genus nolin (Nolina Michauz.) Or so-called "bottle tree". This representative of the "hard-leaved" succulents has narrow, long and hard leaves that are not a water store. The water in these plants is stored in an expanded stem base or caudex reservoir, and is used in case of drought. In our greenergy, this plant appeared in the 1980s. Today it grows in the subtropics division, its height reaches about 2 meters, the length of the leaves is 1.5 meters, and the diameter of the caudex is 1 m. Hats, mats and other items are woven from the leaves of *Nolina* in the homeland.

The spurge family (Euphorbiaceae) are stem succulents. Our collection includes 3 genera (Euphorbia L., Monadenium Pax. And Syandenium Boiss.), 11 species. The leaves of these plants appear exclusively at a young age, and then they fall off, since photosynthesis occurs directly in the stems. Here they store water, which, in case of its shortage, is used. The juice of these plants is poisonous, so you need to work with them carefully.

Picture 3 – *Euphorbiaobesa* Hook

Thus, as a result of the introduction work, promising and commercially beneficial plants from the succulents of our collection were chosen, since they are not whimsical in care, and as a result of evolution can easily adapt to a more or less arid climate, can be widely used. In this publication, we talked about the genera of succulents, interesting for phytodesign.

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СУККУЛЕНТНЫЕ РАСТЕНИЯ В КОЛЛЕКЦИИ ИНСТИТУТА БОТАНИКИ И ФИТОИНТРОДУКЦИИ Г. АЛМАТЫ

Аннотация. «Алматы қаласының Ботаника және фитоинтродукция институтының жинағындағы суккулентті өсімдіктер» мақаласы біздің коллекцияның сукуленттерімен таныстыру жұмыстарының нәтижелерін ұсынады. Болашағы бар, сатуға қолайлы Суккулентті өсімдіктер таңдалып алынған, өйткені олар күтімді қатты қажет етпейді және эволюция нәтижесінде құрғақ климатқа жеңіл бейімделгендіктен, кеңінен қолданысқа ие болады. Бұл жарияланымда біз фитодизайн үшін қызықты әрі құнды суккуленттердің туыстары жайында айтамыз.

Түйін сөздер: суккулент, интродукция (жерсіндіру), жабық грунт, коллекция, фитодизайна.

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СУККУЛЕНТНЫЕ РАСТЕНИЯ В КОЛЛЕКЦИИ ИНСТИТУТА БОТАНИКИ И ФИТОИНТРОДУКЦИИ Г. АЛМАТЫ

Аннотация. В статье «Суккулентные растения в коллекции Института ботаники и фитоинтродукции г.Алматы» приводится результаты интродукционной работы с суккулентами нашей коллекции. Были выбраны перспективные и коммерчески выгодные растения из суккулентов, так как они не прихотливы в уходе, а в результате эволюции легко адаптируются к более или менее аридному климату, могут широко использоваться. В данной публикации мы рассказали о родах суккулентов, интересных для фитодизайна.

Ключевые слова: Суккулент, интродукция, закрытый грунт, коллекция, фитодизайна.

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