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Nidoviruses associated with aquatic animals

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SUMMARY

Nidoviruses comprise the most complex grouping among other viruses with respect to their multiplicity, phylogeny, systematics, species identification, genetic relationships within a taxon, progressive variability. Like other single-stranded RNA viruses, nidoviruses have a relatively high ability to mutate and recombine, which allows them to quickly adapt to new hosts and new ecological niches. Although most of the known representatives of nidoviruses are associated with terrestrial hosts, more and more data has recently appeared on nidoviruses recovered from aquatic organisms. This review is the analysis of current data on the representatives of the order *Nidovirales* associated with aquatic animals. They are all included in the eight families based on the current classification of viruses. The most studied among them are members of the families *Coronaviridae*, *Tobaniviridae* and *Roniviridae*. Representatives of the other families of aquatic animal nidoviruses were identified using metagenomic deep sequencing (metagenomics), but their effect on the host organism has not yet been adequately studied. Data on the distribution of nidoviruses among aquatic animals in different global aquatic systems are presented, clinical signs of the disease are described, a brief description of nidoviruses and their genomes is given. Nidoviruses of aquatic animals as the earliest members of the animal kingdom are supposed to have played a possible role in the evolution of terrestrial animal nidoviruses. Therefore, aquatic animal nidoviruses could play a significant role in the formation of new natural reservoirs unknown to science, as well as in their interspecies transfer between marine, freshwater and terrestrial hosts.

Key words: nidoviruses, coronaviruses, tobaniviruses, roniviruses, spread, pathogenesis.

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Нидовирусы, ассоциированные с водными животными

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РЕЗЮМЕ

Нидовирусы в отношении их многочисленности, филогенеза, систематики, видовой идентификации, генетических связей внутри таксона, прогрессивной изменчивости являются наиболее сложной группировкой среди прочих вирусов. Как и другие вирусы с односпиральной РНК, нидовирусы обладают сравнительно высокой способностью к мутациям и рекомбинациям, что позволяет им быстро адаптироваться к новым хозяевам и новым экологическим нишам. Хотя бóльшая часть известных представителей нидовирусов ассоциирована с наземными хозяевами, в последнее время появляется все больше сведений о нидовирусах, изолированных из водных организмов. В обзоре анализируется современная информация о представителях отряда *Nidovirales*, ассоциированных с водными животными. Согласно современной классификации вирусов все они входят в состав восьми семейств. Наиболее изученными среди них являются члены семейств *Coronaviridae*, *Tobaniviridae* и *Roniviridae*. Представители остальных семейств нидовирусов водных животных были выявлены методом углубленного секвенирования (метагеномики), но их влияние на организм хозяев пока изучено недостаточно. Приведены данные по распространению нидовирусов среди водных животных в различных водных системах мира, описаны клинические признаки заболевания, дана краткая характеристика нидовирусов и их геномов. Предполагается возможная роль нидовирусов водных животных, как наиболее древних представителей животного мира, в эволюции нидовирусов наземных животных. Поэтому нидовирусы водных животных могут иметь большое значение для установления новых, неизвестных науке природных резервуаров, межвидового их переноса между морскими, пресноводными и наземными хозяевами.

Ключевые слова: нидовирусы, коронавирусы, тобанивирусы, ронивирусы, распространение, патогенез.

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Nidoviruses (*Nidovirales*) are the order of enveloped viruses with a single-segment linear single-stranded positive-sense (+)RNA. They comprise the most complex grouping among other viruses as regards their multiplicity, phylogeny, systematics, species identification, genetic relationships within a taxon, progressive variability. As obligate parasites, nidoviruses form parasitic systems with reservoir hosts belonging to all relevant veterinary and medical categories – food-producing and small domestic animals, humans, synanthropes, rodents, bats, wild animals, birds, fish.

The spectrum of pathogenicity and epidemiological significance of nidoviruses varies from fairly balanced relationships of mutual tolerance within the parasite-host system, when viruses remain "orphan" and do not cause specific pathology, to severe, fatal, nosologically determined epidemic infections such as porcine transmissible gastroenteritis (TGEV), feline infectious peritonitis (FIPV), bovine winter dysentery, avian infectious bronchitis, severe acute respiratory syndrome and Middle East respiratory syndrome passing through a series of intermediate phenomena at the clinical and epidemic levels. Speaking of the latter, the probability of the development of factorial, conditionally dependent pathology in the form of pneumoenteritis resulting from stressful effects on the host organism with a decrease in its resistance, provoking a parasitic system imbalance that turns the host carrier into an active source of infection, needs serious attention [1, 2].

Although most of the known nidoviruses are associated with terrestrial hosts, more and more information has recently appeared on nidoviruses isolated from aquatic organisms. Some aquatic animal nidoviruses have been recently identified by metagenomic deep sequencing, but their effect on the host organism has not yet been sufficiently

Table
Nidoviruses associated with aquatic organisms

Таблица
Нидовирусы, ассоциированные с водными организмами

Family	Genus	Type species	Host
Coronaviridae	*Alphacoronavirus*	*Harbor seal coronavirus* (HSCoV)	Seal
	Gammacoronavirus	1. *Beluga whale coronavirus* (SW1) 2. *Bottlenose dolphin coronavirus* (BdCoV)	Beluga whale Dolphin
	Alphaletovirus	*Microhyla alphaletovirus 1* (MLeV)	Microhyla*
Tobaniviridae	*Bafinivirus*	1. *White bream virus* (WBV) 2. *Fathead minnow nidovirus* (FHMNV)	White bream Minnow
	Oncotshavirus	1. *Chinook salmon nidovirus* (CSBV) 2. *Crucian carp nidovirus (CCNV)*	Chinook salmon Crucian carp
Roniviridae	*Okavirus*	1. *Gill-associated virus* (GAV) 2. *Yellow head virus* (YHV) 3. *Palaemon nidovirus* (PAN)	Shrimp Shrimp Swimming crab
Euroniviridae	*Charybnivirus*	1. *Charybnivirus* (CharNV) 2. *Decronivirus* (DecNV)	Crab Shrimp
	Paguronivirus	*Paguronivirus* (PagRV)	Pagurus crayfish
Mononiviridae	*Alphamononivirus*	*Planidovirus 1* (PSCNV)	Planaria
Mesoniviridae	*Alphamesonivirus*	*Alphamesonivirus 1* (NDiV)	Mosquitoes*
Abyssoviridae	*Alphaabyssovirus*	*Aplysia abyssovirus 1* (AAbV)	Mollusk
Medioniviridae	*Bolenivirus*	*Botrylloides leachi virus*	Tunicates

* Animals living in the aquatic environment only at the larval stage.

* Животные, обитающие в водной среде лишь на личиночной стадии.

Fig. 1. Pacific seals (Phoca vitulina richardsii) on the Central California coast (https://specials-images.forbesimg.com/imageserve/1170425445/960x0.jpg?fit=scale)

Рис. 1. Тихоокеанские тюлени (Phoca vitulina richardsii) на побережье Центральной Калифорнии

studied. In this regard nidoviruses of aquatic animals, as the earliest members of the animal kingdom could play a significant role in the formation of new natural reservoirs unknown to science, as well as in their interspecies transfer between marine, freshwater and terrestrial hosts.

According to the current classification of viruses (International Committee on Taxonomy of Viruses (ICTV), 2019), all novidoviruses associated with aquatic organisms belong to eight families (See the Table). The most studied among them are members of the families *Coronaviridae*, *Tobaniviridae* and *Roniviridae*. The representatives of other families of aquatic animal nidoviruses have not been adequately studied.

Harbor seal coronavirus was first reported in the United States in 1987 in three common seals (*Phoca vitulina*) housed at the Seaquarium in Florida [3]. One animal exhibited leukocytosis, accompanied by dehydration and hyperchloremia, and two seals died without showing any clinical signs. The pathological examination of all three animals revealed extensive bronchoalveolar hemorrhages with severe diffuse pulmonary congestion. The spleen, visceral and peripheral lymph nodes were characterized by lymphoid depletion. Using immunofluorescence staining method and antisera against various coronaviruses, it was shown that positive results were observed only using antisera against alpha-coronaviruses (TGEV, FIPV, CCoV – canine coronavirus enteritis) and negative results were obtained when the antisera against bovine beta-coronavirus (BCoV) were used. Based on that, it was decided to assign this virus (HSCoV) to the genus *Alphacoronavirus*. A short nucleotide sequence of harbor seal coronavirus has been published in GenBank (NCBI, Acc. No. FJ766501) (https://www.ncbi.nlm.nih.gov/nuccore/FJ766501.1/).

In June 2000 twenty-one individuals in a herd of resting Pacific seals (*Phoca vitulina richardsii*) were found dead on the central coast of California (Fig. 1) [4].

Three different viruses were isolated from pathological material obtained from the dead seals. Coronavirus was identified in five animals using polymerase chain reaction (PCR). The biopsy of the dead seals revealed pulmonary stagnation and extensive hemorrhages.

Coronavirus of beluga whale (*Delphinapterus leucas*) belonging to the family *Monodontidae*, order *Cetacea* and found in all coastal waters of the Arctic seas, including the White, Bering, Okhotsk, and occasionally Baltic seas, was first identified in the USA in 2008 and was named SW1 [5]. A captive-born male beluga whale died from acute liver failure after suffering an acute generalized pulmonary disease. Histological examination revealed numerous red-yellow hepatic necroses (Fig. 2).

Electronic microscopic examination of liver tissue revealed a great number of round viral particles 60–80 nm in diameter in infected tissues. Attempts to isolate the pathogen in continuous cell lines failed. The viral genome was approximately 31,700 nucleotides and encoded nonstructural (ORF1 and ORF1b) and structural (ORF2, ORF3, ORF4 and ORF11) proteins. The SW1 nucleotide sequence was deposited in GenBank (NCBI, Acc. No. EU111742). The deduced amino acid sequences showed no similarity to proteins from known coronaviruses in 2008. At present its close relationship with Bottlenose dolphin coronavirus has been established [6].

Coronavirus of bottlenose dolphin (*Tursiops truncatus*), inhabiting temperate and warm waters of the World Ocean, as well as the Mediterranean, Baltic and Black Seas, was discovered by Chinese researchers in 2014 [6]. The

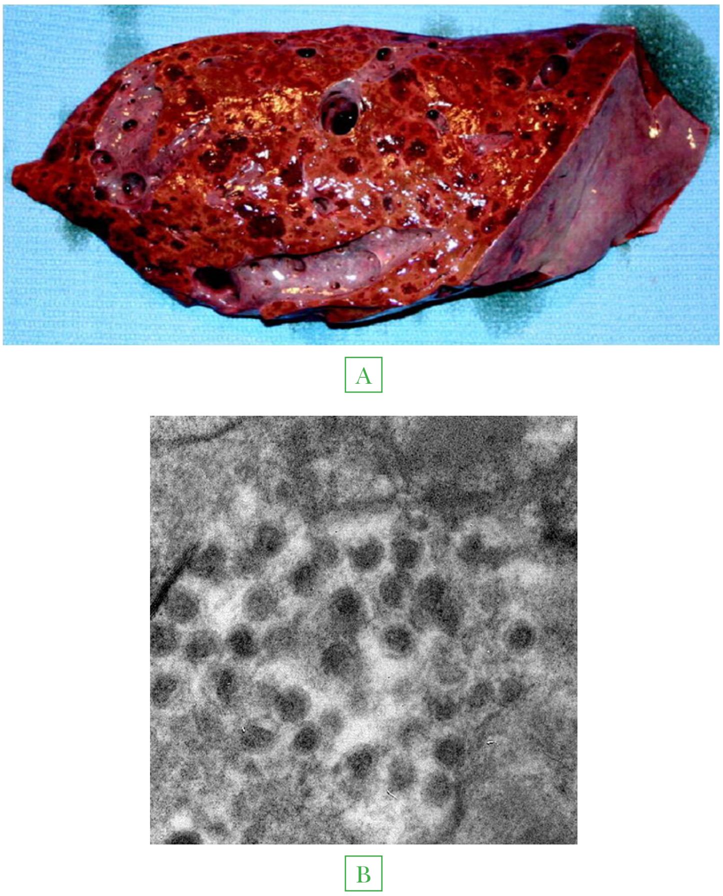

Fig. 2. A. Necrosis areas in the liver of the beluga whale affected by SW1 coronavirus.
B. The aggregates of spherical viral particles in infected tissues [5] (https://jvi.asm.org/content/jvi/82/10/5084/F1.large.jpg)

Рис. 2. A. Некрозы в печени белухи, пораженной коронавирусом SW1.
B. Скопления сферических вирусных частиц в инфицированных тканях [5]

virus was named BdCoV. Complete genome sequencing showed that BdCoV is closely related to Beluga whale coronavirus (SW1) and they both belong to gammacoronaviruses.

The BdCoV genome is 32,000 nucleotides and in 2014 it was the largest among all known coronaviruses. The large size of its genome is a result of multiple unique open reading frames (NS5a, NS5b, NS5c, NS6, NS7, NS8, NS9, NS10) located between the M and N genes. The major difference between the two abovementioned coronaviruses was observed in the proteins encoded by their spike (S) genes [6].

Microhyla alphaletovirus 1 (MLeV) was detected by metagenomic analysis of the common intracellular RNA pool of the ornamented pygmy frog *Microhyla fissipes* [7]. This frog is up to 3–4 cm in size and is widespread in many countries of Southeast Asia. The virus genome contains 22,304 nucleotides, the nucleotide sequence is available in GenBank (Acc. No. GECV01031551). In the process of frog metamorphosis the number of viral transcripts decreases by 7–14 times; they are not detected at all in adult frogs [7].

Carp fish bafiniviruses are classified in a separate genus (*Bafinivirus*), which is included in the family *Tobaniviridae*. The genus name is made up of the first two letters of the English words '**ba**cilla', '**fi**sh' and '**ni**dovirus' and is due to the fact that the virus has a bacilliform shape (Fig. 3).

The fish bafinivirus was first discovered in grass carp (*Ctenopharyngodon idella*) by German ichthyopathologists in 1987 [8]. Grass carp has been introduced to many countries and now ranks first in the world in terms of commercial rearing. The virus was reported in apparently healthy grass carp from Hungary during cross-border veterinary control. The virus isolate multiplied in CAR (continuous) cells (goldfish [*Carassius auratus*] fin cells), CLC cells (common carp [*Cyprinus carpio*] leukocytes) and FHM cells (fathead minnow [*Pimephales promelas*] caudal stem cells) at 15–25 °C. The infected cells fused, then lysed. Electron micrographs of affected cells revealed that the virus particles were bacilliform in shape measuring 170–220 nm in length and 50–55 nm in diameter. Staining with an orange acridine solution showed that the virus contains a single-stranded RNA. The virus is inactivated by chloroform, acid solutions (pH 3) and at a temperature of 56 °C.

A year later, a similar virus was reported in Japan during an outbreak of acute infection of common carp (*Cyprinus carpio*). Affected individuals demonstrated erythema on the abdomen and hepatic and renal necrosis. The virus is transmitted to carp fry via the water at a temperature of 20 °C. The bafinivirus was described in color carp (*C. carpio haematopterus*) in the same country, during the study of the disease named 'ana-aki-bio' [9]. Developed lesions were observed in visceral organs, the virus particles were identified in hematopoietic tissue and spleen. Infected epithelioma papillosum cyprin (EPC) cells displayed karyopyknosis and intracytoplasmic vacuolization.

The bafinivirus of white bream, or silver bream (*Blicca bjoerkna* L.), a fish species which is widespread in the basins of the Baltic, Black and Caspian seas, in the European and Caucasian countries, was first detected in Germany during studies of fish health in the natural environment [10]. The virus is named WBV in the English-language sources. The virus does not induce visible pathological changes in the white bream body, its effects are manifested only in continuous fish culture cells.

WBV particles are bacilliform in shape (130–160 nm in length and 37–45 nm in diameter). The virion is covered with a lipid envelope with surface projections (20–25 nm in length). The polyadenylated WBV RNA has five genes encoding open reading frame: ORF1a, ORF1b, ORF2, ORF3 and ORF4. The ORF1a/1b gene encodes pp1a and pp1ab polyproteins containing proteinase, polymerase and other replication enzymes common to all nidoviruses, and ORF2, ORF3, and ORF4 are the surface projection glycoprotein (S), membrane protein (M), and nucleocapsid (N) protein, respectively [11].

Fat-minnow bafinivirus (*Pimephales promelas*) was discovered in the USA in 1997 [12]. Minnows (*Phoxinus*) are the genus of small (not larger than 20 cm) freshwater fish in the family *Cyprinidae*. The virus is named *Fathead minnow virus* (FHMNV). It replicates in the EPC, FHM and RTG (rainbow trout gonads) cells at 15–25 °C. Syncytium is produced in FHMNV-infected cells.

The disease of the fathead minnow becomes evident by behavioral changes characterized by fish initially swimming erratically in circles before becoming listless either at the tank bottom or with their head orientated upward at the water surface. The course of the disease is consistent with the temperature. In 19 °C water mortalities accumulate rapidly from 3 days post-challenge, but 17 °C water delays mortality onset to 13 days post-challenge. Hemorrhages are observed on the skin as well as in the liver, kidneys and spleen of infected individuals. Hemorrhages in the muscles may also be evident and the kidneys in many fish may become visible due to musculature edema and darkening of the trunk kidney. FHMNV is highly host-specific; channel catfish, goldfish and golden shiners are not FHMNV susceptible [12].

FHMNV virions are bacilliform in shape (130–185 nm in length and 31–47 nm in diameter). The complete nucleotide sequence of the virus has been established [11]. The virus single-stranded RNA contains 27,000 nucleotides and is similar to white bream coronavirus. Phylogenetic analysis using the conserved region in the gene helicase motif of FHMNV showed that it is most closely related to WBV. A comparison of the gene products of helicase (pp1ab domain), S, M, N, and ORF1ab with the WBV gene products shows different levels of homology ranging from 15 (protein S) to 70% (helicase) [13].

Chinook salmon (*Oncorhynchus tshawytscha*) **nidovirus** was discovered in Canada in 2014 [14]. The virus is named CSBV. It replicates and induces cytopathic effects in RTG-2 and EPC cells at the temperatures of 15, 20 and 25 °C. Viral particles are rod-shaped, 45 nm in diameter and 120–130 nm in length. The genome of the virus contains 27,004 nucleotides and the organization of genes corresponds to that of nidoviruses. The complete nucleotide sequence is available in the GenBank database (NCBI) (Acc. No. KJ681496). Based on the amino acid composition, this virus was found to be related to WBV and FHMNV [15].

Another salmonid virus was detected by Canadian researchers in Atlantic salmon (*Salmo salar*) [15]. The virus is named ASBV. Analysis of the complete nucleotide sequence of its genome revealed that it is 99% identical to Chinook salmon coronavirus (CSBV). However, it has a large deletion in the pp1a replicase polyprotein gene. Genome sequence analysis also revealed the alleged sixth protein, which may be the envelope protein [15, 16]. A broad spectrum of sensibility of continuous cell cultures to this virus may indicate a wide range of its hosts in the natural environment.

Crucian carp or goldfish (*Carassius auratus*) **nidovirus** was first described by Chinese researchers in 2019 [17]. The virus is named CCNV (*Crucian carp nidovirus*). The CCNV genome contains 25,971 nucleotides, it has five open reading frames encoding 1ab polyprotein (pp1ab), peplomer glycoprotein (S), membrane protein (M) and nucleocapsid protein (N). By the organization of the genome, this virus is related to Chinook salmon nidovirus.

Shrimp okavirus. Shrimp nidoviruses belong to the genus *Okavirus* in the family *Roniviridae*. The family name combines two words that designate the form of the virion (**ro**d-shaped) and the taxonomic name of the order (***Ni**dovirales*). The name of the genus *Okavirus* comes from the name of the shrimp lymphoid organ (in English 'oka'), in which the virus is most commonly detected. Okaviruses comprise a complex group consisting of six genotypes. Genotype 1 is the YHV shrimp okavirus that causes a disease called "yellow head" affecting tiger shrimp (*Penaeus monodon*); genotype 2 includes shrimp gill-associated okavirus (GAV); viruses of genotypes 3–6 are low pathogenic and do not cause symptoms or signs of the disease.

Yellow head disease (YHD) was first reported in Thailand in giant tiger shrimp. The disease onset is announced by a rapid abnormal increase in feed consumption followed several days later by an equally rapid cessation in feeding and swelling of the digestive glands. A day later infected individuals begin to swim erratically and restlessly at the water surface [18]. YHV can affect shrimp and other species, as well as some krill species. To date the virus has been detected in Southeast Asia, Australia, America, and East Africa [19, 20]. YHV leads to 90–100% shrimp mortality within 3–5 days after the onset of the first symptoms. It was commonly observed that diseased shrimps had bleached or yellowed gills and pale-yellow hepatopancreas (paired structures in the small intestine of invertebrates functioning as both the pancreas and liver). One more day later the shrimp cephalothorax becomes intensely yellow. Then the number of affected individuals increases sharply, leading to almost complete population losses within three days after the onset of symptoms. The YHV virus can remain viable in water at 25–28 °C for about 4 days after the infection resolves.

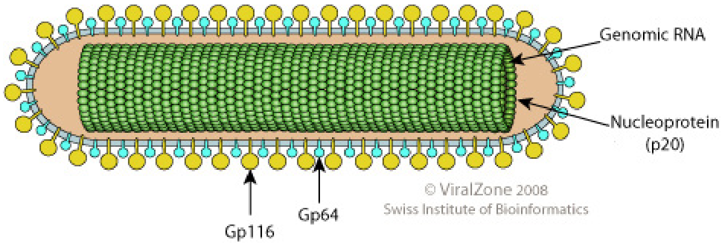

Fig. 3. Schematic structure of bafiniviruses (https://viralzone.expasy.org/resources/Roniviridae_virion.jpg)

Рис. 3. Схема строения бафинивирусов

Shrimp larvae weighing 5–15 g are the most susceptible to GAV ovirus [21, 22]. Adult shrimps are also susceptible to the virus. Coronavirus infection occurs when shrimps scavenge on dead individuals or directly via contaminated water and infected nets or tools. The disease can be both acute and chronic, the pathogen is transmitted both horizontally and vertically. Shrimp mortality can be significant in case of acute-stage infection, and the virus can invade all tissues of mesodermal and ectodermal origin, most often – the lymphoid organ. Basophilic cytoplasmic inclusion bodies can be observed in necrotic cells.

Shrimp okavirus virions have an outer envelope and are rod-shaped with rounded ends measuring 40–60 × 150–200 nm. The envelope is covered with spikes (peplomeres) protruding about 11 nm above the surface of the virion. Nucleocapsids are 20–30 nm in diameter and appear as a coiled filament with a 5–7 nm periodicity. Long filamentous nucleocapsid precursors (approximately 15 nm in diameter and 80–450 nm in length) occur in the cytoplasm of infected cells, where they acquire envelopes by budding on the membranes of the endoplasmic reticulum. Newly formed mature virions often appear in the form of congregations, as a result of which paracrystalline arrays may be visualized.

Shrimp okaviruses contain one linear segment of a single-stranded positive-sense RNA, the length of which varies from 26,235 nucleotides for the GAV virus to 26,662 nucleotides for YHV. The YHV okavirus ORF4 is significantly shorter as compared to that of other known genotypes, and cannot always be expressed. The GAV genome complete sequence and YHV genome partial sequences are available [21].

Along with the *Okavirus* genus, a new genus only represented by the nidovirus isolated from the Chinese mitten crab *Eriocheir sinensis* is supposed to be assigned to the family *Roniviridae* [23]. This crab is a dangerous invasive species that has spread from the Yellow Sea to many European and North American countries. It is found in the Karelian reservoirs and the Volga River. The crab nidovirus (EsRNV, *Eriocheir sinensis ronivirus*) causes the so called "sighs disease" because affected crabs make sounds similar to sighs at night. EsRNV virions are rod-shaped, 16–18 nm in diameter and 15–20 nm, occasionally up to 400 nm, in length. Experimental infection of crabs resulted in 100% mortality within 13–17 days. The viral particles are found in the connective tissues of many organs, including gills, hepatopancreas, heart, intestines, and ovaries [23].

The genomes of the members of the family *Euroniviridae* were studied using bioinformatic methods, but the biological properties of these nidoviruses are not yet well known.

Planaria nidovirus was discovered at the end of 2018 in the Mediterranean planaria *Schmidtea mediterranea* [24]. This is a type of flatworm that lives in fresh water on the islands of the Mediterranean Sea, in Spain and Tunisia, and is particularly useful for studying regeneration processes. Planaria nidovirus was detected by metagenomic analysis of a common pool of intracellular RNA. The virus was named *Planarian secretory cell nidovirus* (PSCNV). It is propagated in planarian secretory cells. The electron microscope examination demonstrated presence of spherical, slightly elongated virus particles, associated with the endoplasmic reticulum membranes, 90–150 nm in diameter, in the cytoplasm of affected cells. The PSCNV genome contains 40,671 nucleotides [24]. It is currently the largest genome among all known RNA viruses.

The main difference between PSCNV and other nidoviruses is the presence of an unusually large reading frame ORF1b in its genome. It is suggested that PSCNV diverged early from nidoviruses and acquired additional genes typical of large DNA viruses, namely genes encoding ankyrin and fibronectin. Newly acquired genes may significantly affect the virus-new host interaction [24].

Mosquito nidovirus was detected in Vietnam by metagenomic analysis from a pool of blood-sucking mosquitoes of the genus *Culex* [25]. The virus was named Nam Dinh (NDiV) due to the name of the area where mosquitoes were collected for research. It does not cause pathological changes in mosquitoes. The virus replicates in the continuous mosquito cell line C6/36 derived from the *Aedes albopictus* members. Virions are spherical, 60–80 nm in diameter. The virus genome contains 20,192 nucleotides and has 5 open reading frames. Based on its genomic structure this virus was assigned to a separate family *Mesoniviridae*, which is in transition from "large" to "small nidoviruses" (*Arteriviridae*).

Nidovirus of aplysia, or sea hare (*Aplysia*), one of the largest herbivorous mollusks reaching 1 m in length and living in warm and subtropical seas, was simultaneously described by two independent research groups [7, 26]. Like the Planaria virus, the Aplizia nidovirus was also detected in the gastropod mollusk *Aplysia californica* by metagenomic analysis of the common pool of intracellular RNA. The BLAST bioinformatics analysis showed that based on its genome structure the virus has properties that are typical for nidoviruses. The virus is named AAbV. The genome contains 35,906 nucleotides, which indicates its belonging to the "large" nidoviruses. The largest amount of virus RNA are found in neurons, as well as it is detected in the gills, salivary glands and muscles. Similar nidoviruses have been described in tunicates (*Botrylloides leachi*), sea snails (*Turritella* sp.) and other aquatic organisms [27, 28].

CONCLUSION

The analysis of the literature available on this topic reveals that the application of the genomic identification principles based on metagenomics gives the possibility of detecting an unpredictable number of new viruses, as it follows from how nidoviruses spread among aquatic animals. The study of the global virosphere significantly expands information on potential reservoirs of emerging pathogens [29]. It is generally recognized that their overwhelming majority are of zoogenic origin [30]. The interest has recently increased significantly in this group of viruses due to the outbreak of severe acute coronavirus infection COVID-19 [31]. Like other single-stranded RNA viruses, nidoviruses have a relatively high ability to mutate and recombine, which allows them to quickly adapt to new hosts and new ecological niches [2, 13, 32]. For instance, SARS-CoV-1-like coronaviruses have been isolated from humans, Himalayan palm civets and raccoon dogs [33]. Phylogenetic analysis of the genomes of various animal nidoviruses suggests that many of them can be descendants of aquatic animal nidoviruses.

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